VOLUME 22 NUMBER 1

JANUARY, 1932

Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

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Phytopathological Note 103

Price { $6.00 per year, United States and dependencies, Mexico and Cuba
$6.25 per year, Canada
$6.50 per year, elsewhere

PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

COR. LIME AND GREEN STREETS, LANCASTER, PA.

Entered at the post-office in Lancaster, Pa., as second-class matter.

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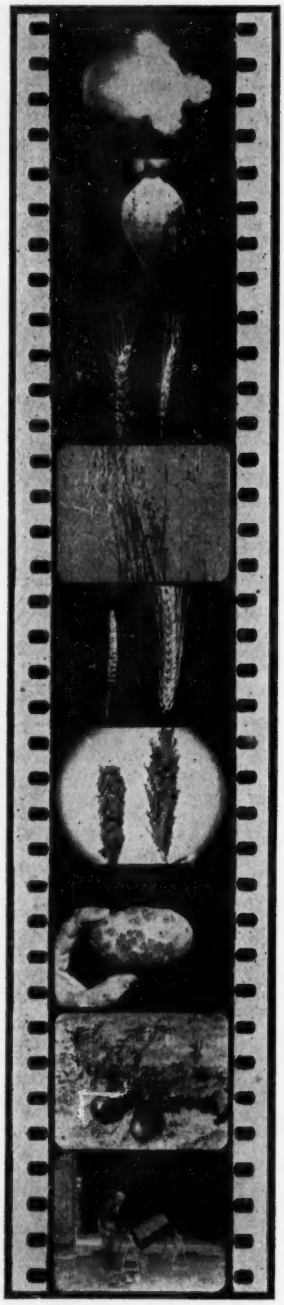

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Advertisements. Rates may be secured from business management. No indorsement of any statement of claims made in advertisements is assumed by this journal or by The American Phytopathological Society.

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Causes and control of damping off of tomato seedlings. L. J. ALEXANDER, H. C. YOUNG, and C. M. KIGER.

Two fungi, *Pythium ultimum* and *Rhizoctonia solani* Kühn (*Corticium vagum* B. & C. var. *solani* Burt), have been found to be the causes of damping off of tomato seedlings, in greenhouses. Inoculation experiments demonstrated that *P. ultimum* caused the major loss. The symptoms of the two diseases are similar but differ in that *R. solani* causes malformations of the cotyledons and produces a drier type lesion than *P. ultimum*. In soil-temperature and soil-moisture experiments *P. ultimum* was least destructive at 30° C. and was most destructive between 18° and 24° C. Dry soil was found unfavorable to the development of both diseases. Soaking tomato seed in any of several strengths of copper sulphate solution increased the percentage of emergence but, later, the seedlings damped off severely. In sterilized soil, to which pure cultures of either *P. ultimum* or *R. solani* had been added, excellent control of the disease was secured by disinfecting it with a formaldehyde dust. The dust was prepared by adsorbing 15 parts by weight of 40 per cent commercial formaldehyde on 85 parts of a carrier composed of 1 part of Kaolin and 2 parts of diatomaceous earth. The best control was secured when 42 grams of the dust was mixed with each square foot of seed bed soil, which was 2½ to 3 inches deep. Immediately after treating, seed was sown and the soil was thoroughly wetted. Several other chemical mixtures were tried but gave unsatisfactory control of damping off.

Canker of black walnut caused by a Nectaria sp. J. M. ASHCROFT.

A survey of West Virginia has located the canker of black walnut reported by Orton (Science 72: 142–143. 1930) in 21 counties of the State. This disease has been observed also in Pennsylvania and Virginia, and specimens have been received from Rhode Island, Wisconsin, and Ontario. Reports have been received of its presence in 6 other counties of West Virginia as well as in Tennessee and North Carolina.

A Nectria is almost invariably associated with the canker. Inoculations were made by inserting conidia from pure cultures of this organism into wounds made in the bark of old and young black-walnut trees. Out of 104 inoculations, made between February 18 and April 28, 79 per cent produced the symptoms of the disease. None of the controls showed such symptoms. The fungus has been repeatedly reisolated from the artificially induced cankers.

The characteristic ringed appearance of the canker results from a seasonal alternation of the dominance of the fungus growth with that of the host. The period of active growth of the fungus is late winter and early spring. All regions of the trunk are invaded by the fungus.

Studies of the fungus in culture and from natural sources reveal a close relationship with *Nectria ditissima*.

The resistance of certain varieties of grapes to Phymatotrichum root rot. WALTER J. BACH and J. J. TAUBENHAUS.

Field inoculations at Substation No. 15 with *Phymatotrichum omnivorum* on grapes during 4 seasons have shown a difference in degree of susceptibility of varieties. Several varieties, which appear to be desirable as rootstocks, show a high degree of resistance. Approximately 104 varieties and 12 of the most promising rootstocks were included in the test. During the 1930 and 1931 seasons, in addition to the inoculation of the individual vines, cotton, a very susceptible host, was interplanted in the rows and also inoculated. The varieties that have withstood this test so far and also appear to be

vigorous enough for promising rootstocks are: Champanel, Mustang, Vitis Constancia, V. Salonis, Dog Ridge, V. Berlandieri, V. Champini, and Black Spanish. Excavations of grape root systems have revealed the presence of mycelium of the root-rot fungus on the roots near the points of inoculation on varieties that appear to be resistant. Since some varieties of grapes are very susceptible, the facteor of resistance must differ for varieties.

The pathogenicity of Bacterium translucens var. undulosum. R. H. BAMBERG.

In addition to wheat, rye, barley, and speltz, *Bacterium translucens* var. *undulosum* infects oats, *Hordeum jubatum,* and *Bromus inermis.*

Cultures obtained from rye readily infected wheat seedlings and produced typical black-chaff lesions. Cultures obtained from barley with lesions similar to those previously described for *Bacterium translucens* also produced lesions on wheat seedlings typical of *Bact. translucens* var. *undulosum.*

The pathogenicity of cultures isolated from wheat from different localities varied considerably. Four variants arising as sectors differed from the parent cultures both culturally and in pathogenicity. Lesions appeared on inoculated wheat seedlings in 48 hours at about 24° C. (ordinary greenhouse temperature), but not until after 20 days at 10° C. In the field in 1931, wheat plants were readily infected early and late in the growing season but not during the heat of midsummer. Symptoms vary greatly with environmental conditions. Wheat plants in the boot stage, inoculated with *Bacterium translucens* var. *undulosum,* became infected on the leaves, awns, glumes, rachides, and necks. Infected areas on plants in the greenhouse became typically black, while most of those on plants kept at 10° and 20° C., under artificial light, remained yellowish and water-soaked in appearance.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Minnesota Agricultural Experiment Station.)

Apple measles. ANTHONY BERG.

A dematiaceous fungus, isolated in the fall of 1930 from newly formed lesions of apple measles on water sprouts of the season's growth, proved to be the cause of a form of measles that has been very destructive in a large orchard in West Virginia. The fungus grows rather slowly on artificial media, which may account for the failure in previous isolation attempts. Oatmeal and malt-extract agar seem to be best suited to the growth and sporulation of the fungus. On the host, spores are produced apically on stiff, almost seta-like, dark brown, septate conidiophores that arise singly or in loose aggregates from a few pseudoparenchymatous cells on the surface of the dead epidermis. The spores are clavate to obclavate; usually 3-septate or more, olivaceous to almost hyaline. Germination in water is only from basal and apical cells by means of straight, narrow, slow-growing, unbranched germ tubes. Sporulation was found to be most abundant in nature during July and early August, corresponding closely with the period of maximum infection as checked in the field for 2 seasons. In the orchard where the disease was studied Red Astrachan, Maiden Blush, Rome, and Grimes were found to be most susceptible, while Transparent, York, Gano, and Chenango remained free from the disease.

Crown-gall-like hypertrophies on a conifer. J. G. BROWN.

A cypress tree, *Cupressus arizonica,* on the campus of the University of Arizona, was apparently dying from the attack of bark beetles. It was found to have numerous

root galls varying in size from that of a pea to galls several inches in diameter. From these galls a bacterium was isolated, which grew on Patel's medium and caused small galls when inoculated into the tissues of *Ricinus communis*. From the galls on the latter the bacterium was again isolated.

Is Bacterium tumefaciens a mutant or one of the pleomorphic forms of Bacillus radiobacter? NELLIE A. BROWN and LEWIS T. LEONARD.

The frequent appearance of nonvirulent colonies of *Bacterium tumefaciens* on plates made from known crown-gall tissue; the colony and various cultural resemblances between *Bact. tumefaciens* and *Bacillus radiobacter;* the appearance of tumor-like outgrowths on various hosts from which radiobacter was isolated and not *Bact. tumefaciens;* and more especially the appearance of galls ¾ to 1 cm. across in less than 2 weeks on noninoculated cut stems of cowpeas, kept in a moist chamber from which radiobacter was isolated, have contributed to the idea that *Bact. tumefaciens* may be either a mutant, a physiologic form, or one of the pleomorphic forms of *B. radiobacter.*

Bacillus radiobacter was isolated from the cowpea-stem tumors, and various host plants, including cowpeas, were inoculated with it. The cowpea plants were placed under varied conditions of temperature and moisture, but no outgrowths similar to the original ones developed. Filtrates of these cowpea-radiobacter cultures through Chamberland and Berkefeld filters were used alone for inoculations, also filtrates added to cowpea-radiobacter cultures a dissociate form of cowpea radiobacter was used alone for inoculations, also in combination with cultures, but no outgrowths developed. Following other simpler manipulations, one strain produced definite small tumors, 5 to 6 mm. across, on daisy stems.

The effect of leaf rust, Puccinia triticina, on the composition and yield of winter wheats in 1931. R. M. CALDWELL, H. R. KRAYBILL, J. T. SULLIVAN, and L. E. COMPTON.

Yield data and chemical analyses of the plants and grain were secured from replicated plots of severely leaf-rusted and nearly rust-free winter wheats of 8 varieties grown at La Fayette, Ind., in 1931. Nearly rust-free control plants for the comparison were secured by frequent sulphur dusting. Other diseases were almost entirely absent. Separate analyses were made of the vegetative portions and kernels at the early-milk, late-milk, and dough stages and of the mature grain. The rusted plots consistently yielded a lower protein and much less vitreous grain than did the control plots, and, in Fulhard, a hard wheat, a greatly increased percentage of "yellow-berry" kernels. Conversely, the vegetative portions of rusted plants were higher in total nitrogen. Reducing sugars and sucrose were consistently lower in the vegetative portions of the rusted plants. Starch followed the same trend. Significant reductions in yield of grain and straw, test weight per bushel, weight per 1,000 kernels, and number of kernels per head were found in the rusted plots. In Michigan Amber, a susceptible variety, where 4 different degrees of rust severity were secured by variations in dusting procedure, the trends mentioned above were evidenced approximately in proportion to the rust severity.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Purdue University (Indiana) Agricultural Experiment Station.)

Appressorium formation and penetration by leaf rust of wheat, Puccinia triticina, in relation to stomatal aperture. R. M. CALDWELL and G. M. STONE.

By a method of stripping the epidermis from wheat-seedling leaves inoculated with leaf rust, and fixing, staining, and mounting it in absolute alcohol, it has been possible

to study directly the relation of the stomatal aperture at the time of penetration to the entrance of leaf rust into its host. Appressoria, substomatal vesicles, and infection hyphae are clearly evident in such preparations. Closed stomata offer no impediment to penetration of wheat seedlings by this rust. These studies indicate, rather, that the formation of an appressorium over an open stoma stimulates it to close tightly prior to penetration by the rust. This response of the guard cells would make penetration impossible. These observations on epidermis strips have been checked by similar findings on the living plants. Often a small stomatal slit is evident between the appressorium and the substomatal vesicle, apparently resulting from the penetration tube pushing between the guard cells. Inoculations with leaf rust at different temperatures had no effect in altering this relation to stomatal aperture.

Uromyces fallens, when inoculated onto wheat leaves, penetrated abundantly and behaved identically with *Puccinia triticina* in its relation to the stomata.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Purdue University (Indiana) Agricultural Experiment Station.)

Cultural races of Pestalozzia funerea and the production of variants resembling Monochaetia. CLYDE CHRISTENSEN.

Fifteen races of *Pestalozzia funerea* were obtained by isolating 150 individual conidia from acervuli borne on the needles of long-leaf pine, *Pinus palustris.* These races were distinguished from each other by the following cultural characters: rate of growth; amount of surface and aerial mycelium; color, topography, and zonation of the colonies; and abundance, distribution, and size of acervuli, and time required for their production. In addition, the spores of the different races varied in size, color, shape, and in length and number of setae. Ten variants, differing from their parents and each other in the characters listed above, arose in the form of sectors in cultures of the races isolated from pine needles. Races conforming to the description of Monochaetia were obtained from conidia isolated from pine needles and arose also as variants in monoconidial cultures that normally produced spores bearing 3 to 5 setae. Seven species of conifers were inoculated with spores of the different races, but none were parasitic under the conditions of the experiment.

Physiologic specialization in Helminthosporium gramineum. J. J. CHRISTENSEN and T. W. GRAHAM.

The barley-stripe organism, *Helminthosporium gramineum,* consists of numerous cultural and parasitic races. Eighty-four of 226 monosporous cultures, isolated from 76 different collections, were culturally distinct. Forty-nine of these were obtained from Minnesota and the rest from different regions in the United States, Canada, and Germany. With 2 possible exceptions, a distinct cultural race was secured from each collection. Occasionally 2 different races were isolated from a portion of a diseased leaf. The pathogenicity of 75 races was tested on several varieties of barley. The percentage of infection ranged from 0 to 90, some races being very virulent, some moderately so, and others relatively weak. In general, those barley varieties usually susceptible under field conditions were heavily attacked by a large number of races when artificially inoculated. However, some varieties were susceptible to some races but immune from or highly resistant to others. Symptoms vary with the variety and the parasitic race of the pathogen.

Control of powdery mildew of snap beans. HAROLD T. COOK.

Experiments on the control of the powdery mildew of snap beans were conducted at the Virginia Truck Experiment Station in the fall of 1931. Twelve different spray and dust fungicides were tested and, listed in the order of their efficiency, are lime-sulphur spray, Kolofog, Kolodust, sulphur-lime dust, dry lime-sulphur spray, copper-lime dust, Sulfocide, calcium mono-sulphide, Manganar dust, Bordeaux mixture, zinc Bordeaux, and Du Bay 1027TT. An experiment to determine the effect of the time of spraying and the number of applications on control gave the following results. The best control was obtained by spraying 4 times at weekly intervals beginning when the disease first appeared. Fewer sprays beginning at the same time or at later dates gave poorer control. Where series of 2 and 3 sprays were applied, the best results were obtained when the first application was made early.

Rotting of sugar-cane cuttings in Porto Rico. MELVILLE T. COOK.

Thielaviopsis paradoxa is the major cause of the poor germination of sugar-cane seed cuttings in Porto Rico. It is a common and very abundant fungus and attacks many species of plants. It is primarily a wound parasite, penetrating the cut ends and destroying the cell walls of the parenchyma tissue. It causes characteristic reddish discolorations of the tissues, which eventually become black and rotten. The fibro-vascular bundles, which persist after the other tissues are destroyed, can be pulled out of the decaying mass in brush-like bunches. The disease is most severe in the cooler months of the year and in the poorly drained soils. During the summer months it can be found in abundance in the cooler elevated regions.

Zonate eye spot on turf grasses. ARNOLD S. DAHL.

Zonate eye spot, caused by *Helminthosporium giganteum* H. and W., has been common on some turf grasses. The disease occurs on some strains of creeping bent (*Agrostis palustris*) and velvet bent (*A. canina*), which are propagated with stolons. It damages these grasses both in nursery rows and in turf. Under wet conditions the organisms spreads over the leaves of the plants so that entire leaves are killed and the turf is brown. There is a distinct difference in the susceptibility of the various strains of both creeping bent and velvet bent. The Virginia strain of creeping bent, widely grown in the Northern States, is extremely susceptible, while Washington and Metropolitan strains are resistant. Acme and Highland velvet bent are somewhat susceptible, while strain No. 14,276 is resistant. Satisfactory control of this disease was obtained by spraying the turf with solutions of ethyl mercury chloride and other experimental organic mercury compounds, such as ethyl mercury arsenate and phenol mercury acetate. These fungicides controlled the disease only when they were sprayed on the turf and left on the leaves. When the solutions were sprinkled on or when the turf was watered directly after the treatment, they were ineffective. Lime sulphur also checked the disease but was injurious to the grass. Chlorophenol mercury, bichloride of mercury, and calomel were used but did not prove satisfactory.

Relation of axillary-bud development to nodal smut infection in the corn plant. GLEN N. DAVIS.

Eight hundred plants were used to determine the relationship of axillary-bud development and the appearance of nodal smut boils; 400 of the plants were inoculated on June 13 and smut readings taken on August 21. On August 22, 100 plants, each, were injured by removing the ears, tops, or ears and tops, and 100 were held uninjured as checks. Four hundred uninoculated plants were treated likewise. Final smut

readings were taken on September 16. In the inoculated plants nodal infection in the uninjured checks increased 20.5 per cent. from August 21 to September 16. In the same period removal of tops resulted in a 29.0 per cent increase, removal of ears a 63 per cent increase, and removal of both tops and ears a 52.2 per cent increase over the checks. In the noninoculated plants nodal infection in the uninjured checks increased 33.3 per cent in the same period, removal of tops resulted in 43.7 per cent increase, removal of ears 56.2 per cent, and removal of both tops and ears 36.8 per cent increase. Seven out of 10 axillary buds from sweet corn inoculated 10 days previously showed smut mycelium when sectioned. One hundred and ten days after inoculation, 66 per cent of the apparently healthy buds from 10 of the plants showed small mature smut boils when held before a strong light.

A seedling-blight stage of onion bulb rot. GLEN N. DAVIS and CHAS. S. REDDY.

A number of onion growers have been compelled to rotate crops on their old land because of a seedling blight and bulb rot caused by a soil-borne Fusarium. Heavy losses are also sustained in onions grown for sets. In seedlings the tips whiten, die progressively downward and finally disappear. More mature, infected plants show the first symptoms as grayish white tips of the outer leaves. Usually only the upper half of the leaf falls over, but the upright lower half dies and becomes brownish gray. In early stages of seedling infection only 1 of the 3 or more roots, normally sparkling white, appears somewhat dull or lead color and, upon examination and isolation, is found to be parasitized. In older plants, the roots may be found in all stages of decay, finally collapsing. Bulbs rot both in the field and in storage. In plots at Clear Lake only 50 per cent of the original plants remained at harvest and many of these bulbs were diseased. In a large field at St. Ansgar, where maggots and smut were almost completely absent, 90 per cent of the original plants were lost, and the crop was a failure. The organism associated with this disease was sent to Dr. C. D. Sherbakoff for identification and was reported similar to, but not identical with, *Fusarium zonatum* form 1.

The genetics of the smut fungi. SYDNEY DICKINSON.

In the covered smut of oats, *Ustilago kollei*, at least 7 pairs of cultural characters have been found to be segregated in definite ratios at the "reduction divisions." Two of these character pairs are segregated in only 1 ratio, another "color" is found to be segregated in 3 different ratios, while the character pair "type of colony center" is found to be segregated in 5 different ratios. Such segregation ratios suggest that color is the expression of 2 additive linked pairs of Mendelian factors and that type of colony center is the expression of a number of Mendelian factors. The evidence so far obtained is insufficient to show that the relative proportion of these segregation ratios is altered by a change in the external medium, but it is possible that the linkage between the two pairs of color factors is increased by raising the nitrogen-source concentration in the medium. By an extended investigation of the effects of the external medium chiefly on the segregation of 1 character pair, it has been possible to show that the onset and duration—as measured in nuclear divisions—of the process of meiosis is in part controlled by the external environment of the cell.

Pathogenic and cultural comparisons of strains of Rhizoctonia solani. O. H. ELMER.

Pure-line vegetative strains of *Rhizoctonia solani* were compared as to pathogenicity and cultural characteristics. Successive vegetative generations retained the cultural and pathogenic characteristics of their parent cultures. Certain strains differ so widely from others that they can be placed in distinct groups. The group most

frequently encountered produces necrotic lesions on potato stems. Individual strains exhibit distinct differences in cultural characteristics and in virulence. One strain rarely produces potato stem lesions but invests the stem with an abundant, dark mycelial felt. Another strain produces complete necrosis of infected potato sprouts, the infection frequently extending beneath the eye and causing seed-piece decay. Strains of a second group produce superficial, fleck-like lesions on potato stems and cause arrested apical growth of emerging sprouts even in the absence of lesions near the sprout tip. Such plants produce side sprouts whose apical growth may, in turn, be arrested. No evidence has been obtained that the arrest of growth is due to by-products from the parasite. On laboratory media these strains differ considerably from those of the first-mentioned group, producing numerous, small, white, mealy-appearing mycelial aggregations. Other strains occur that do not fall into the above 2 groups.

Growth of Phymatotrichum omnivorum in plant juices as correlated with resistance of plants to root rot. WALTER N. EZEKIEL, J. J. TAUBENHAUS, and J. F. FUDGE.

Phymatotrichum omnivorum has been grown in series of cultures prepared with juices expressed from 4 monocotyledonous plants (corn, onions, cannas, and nut grass), resistant to root rot, and from 4 dicotyledonous plants (cotton, carrots, turnips, and sweet potatoes), susceptible to the disease. The oven-dry weight of the fungus mycelium produced in the cultures was determined. With autoclaved, nondiluted juices, growth of the fungus was markedly inhibited in juices from all the resistant plants, while profuse and heavy growth was obtained with juices from 3 of the 4 susceptible plants. However, with diluted juices from the resistant plants good growth was obtained. Monocotyledonous plants resistant to Phymatotrichum root rot apparently contain materials that, in sufficiently high concentration, can inhibit growth of the root-rot fungus, and the resistance of these plants to the disease probably is based, at least in part, on the presence of such materials.

Concentration of salts and soil reaction as affecting growth of the root-rot fungus in the soil. WALTER N. EZEKIEL, J. J. TAUBENHAUS, and J. F. FUDGE.

Soil-culture studies in the laboratory were undertaken to determine whether the correlation of prevalence and destructiveness of *Phymatotrichum omnivorum* root rot with the soil reaction might be based on the amounts of calcium salts in soils, rather than the pH. In soils in which relatively small amounts of salts had been incorporated, growth of the fungus increased, in general, as the pH of the soil increased. Calcium-carbonate additions to acid soil caused greater increases in growth than the same weights of calcium or potassium added as nitrates, sulphates, or phosphates. This indicates that it was the increased alkalinity rather than additional calcium, potassium, phosphate, or nitrate ions that favored growth of the fungus. The results demonstrated also an inhibitory effect of high concentrations of soluble salts, such as potassium nitrate and phosphate. Toxicity of salts depended on the percentage of the salt in the soil solution rather than in the total weight of soil. Large additions of relatively insoluble salts, such as calcium carbonate and phosphate, did not inhibit growth. Specifically toxic materials, such as potassium carbonate and various disinfectants, inhibited growth even in lower concentrations.

The dissemination of cereal rust spores in the greenhouse by terrestrial invertebrates. A. A. GRANOVSKY and M. N. LEVINE.

The imported garden slug, *Agriolimax agrestis*, and spring tails, *Collembola* sp., have been observed to frequently visit and feed upon uredinia of *Puccinia graminis*.

Plant lice, thrips, fungous gnats, and other insects in feeding also come in intimate contact with the pustules of rusted plants and are found to carry rust spores on their bodies, thus serving as vectors. The rôle of various invertebrates in dissemination of urediniospores of cereal rusts in the greenhouse was determined experimentally. Healthy wheat plants were repeatedly infested with slugs that had previously been allowed to feed on rust pustules. Infections invariably occurred along the slug's trail, marked by mucous secretion. Urediniospores adhere to the secretion on the body of the slug and are thus disseminated from diseased to healthy plants. Infection has been obtained with two physiologic forms of wheat rust by using the slug's intestinal content and excreta as inoculum. The spores that passed through the slug did not lose viability and readily caused infection, although the incubation period was somewhat prolonged. Infection also was obtained by using the common greenhouse thrips, *Heliothrips femoralis*, which were previously allowed to come in contact with urediniospores in Petri dishes or on rusted plants.

(Cooperative investigations between the Division of Entomology and Economic Zoology, the Division of Plant Pathology and Botany, University of Minnesota, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

The sooty-blotch and flyspeck of the apple. A. B. GROVES.

A considerable number of isolations of the sooty-blotch fungus have been made from apple-fruit material since the inception of the present studies 2 years ago. Isolation attempts with the flyspeck fungus have been more limited. Many difficulties have been encountered in the development of an isolation technique that would permit of a high percentage of success. However, a technique has been developed that has given successful results in over 50 per cent of the trials.

Numerous sooty-blotch-thallus types have been observed during these studies, some of which are quite distinct. The types observed varied from those possessing a fine and evenly growing thallus, producing few or no plectenchyma, to types producing very conspicuous plectenchyma with inconspicuous interconnecting mycelium; from those having a finely reticulate thallus to some exhibiting strongly radial growth characters. Types have also been observed that penetrate and make some growth beneath the cuticle. Differences in cultural behavior have been observed in many of the various sooty-blotch isolations. These studies are not complete, however, and possible correlations between the several cultural types and the thallus types, as observed on the apple, await further investigations.

The association of bunt of wheat with loose smut and ergot. W. F. HANNA.

Bunt (*Tilletia levis* or *T. tritici*) and loose smut have been found in the field growing side by side in the same head of wheat. Usually the topmost spikelets contain a few bunt balls, whereas the lower ones are completely destroyed by loose smut. The mortality is high among seedlings inoculated with both *T. tritici* and *Ustilago tritici*. In greenhouse experiments with Kota only 44 per cent of the seeds inoculated with these 2 smuts produced mature plants, as compared with 97 per cent for uninoculated seeds and 74 and 72 per cent for seeds inoculated with *U. tritici* and *T. tritici*, respectively. Of the plants grown from seedlings inoculated with both *T. tritici* and *U. tritici*, 64 per cent produced heads infected only with *U. tritici*, 23 per cent heads infected only with *T. tritici*, and 7 per cent heads infected with the 2 smuts. Young bunted kernels of wheat may become infected with ergot. The upper part of the kernel is converted into bunt spores and the remainder into an ergot body. Kernels of Little Club and

Mindum having an ergot body and a bunt ball fused together in this way have been found.

Physiologic forms of loose smut of wheat. W. F. HANNA and W. POPP.

Two distinct physiologic forms of *Ustilago tritici* have been found in Manitoba. One form, collected on Reward wheat, infects heavily many of the common wheats but failed to infect the durum varieties Mindum and Pentad. The other form was collected on Mindum. The durums are susceptible to this form, whereas the common wheats tested are moderately resistant to it.

An analysis of variation in Botrytis cinerea by single-spore cultures. H. N. HANSEN and RALPH E. SMITH.

Forty-seven strains of fungi of the *Botrytis cinerea* type were isolated from various vegetation. From each of these strains 2 monospore cultures were made. From each of 8 of these, 25 single-spore cultures were made. In most of these strains the growth in all the tubes appeared uniform but in a few considerable variation appeared in regard to presence or absence of sclerotia, production of conidia, color and type of mycelium, and other features of gross morphology. There was much variation between the different strains. In 1 (marked X) 24 (96%) of the cultures (*x*) appeared uniform and like the parent, while 1 (*a*) (4%) varied decidedly in absence of sclerotia and type of mycelium. This strain was studied further in repeated single-spore isolations of successive generations. In each instance 25 monospore tube cultures on potato-dextrose agar were made and new cultures started at 10-day intervals. The test was continued to the F_4 generation. *x* continued uniform throughout. In the F_1 generation *a* separated into 7 (28%) like the parent and 18 (76%) of an entirely new type (*b*). *b* continued uniform to the F_4 generation. *a* separated in the F_2 into 12 (48%) of *a*, 7 (28%) of *b*, and 6 (24%) of a new type (*c*). From this point *b* continued uniform. In the F_3 generation *a* separated into 2 (8%) of *x*, 15 (60%) of *a*, 5 (20%) of *c*, and 3 (12%) of a new type *d*. *c* separated into 1 (4%) of *x*, 14 (56%) of *a*, and 10 (40%) of *c*. Like conditions have been found in the case of species of Phoma, Fusarium, and Ramularia.

Studies of the properties and host reaction of the onion to the yellow-dwarf virus. W. J. HENDERSON.

Yellow-dwarf virus, in sterile distilled water and stored at 29° C., is inactivated after 112 hours, and, in onion leaves stored at same temperature, after 100 hours. Thermal death point of the virus, when heated 10 minutes, lies between 75° and 80° C. Freezing at −10° C. for 10 minutes produced no effect on infectivity of the virus.

Healthy bulbs inoculated in the growing tips with 2 hypodermic injections of 0.75 cc. each, became 80 per cent infected. Three injections of 0.42 cc. each infected 72 per cent and 1 injection of 2 cc. infected 25 per cent of the plants.

Healthy onions, inoculated in leaves when 1½ inches high, expressed symptoms on 35 to 40 per cent of the plants during the current growth, and 50 to 60 per cent had masked expressions in next growth period. Plants 4 to 6 inches in height, when inoculated, expressed from 5 to 20 per cent disease during current growth and 25 to 40 per cent in second growth period. Plants 7 to 8 inches in height, when inoculated, failed to express disease symptoms in the first growth period but expressed 25 to 30 per cent during second.

Further studies on ripe rot of pepper. B. B. HIGGINS.

The causal fungus has been compared to specimens and cultures of *Vermicularia capsici* from India and the two have been found to be identical. Pimiento fruits inoculated with a culture of *V. capsici* received from India developed symptoms identical with the ripe-rot symptoms previously observed here. Cultures of morphologically-similar fungi from a number of other host plants have all proved either nonparasitic or weak wound parasites on Pimiento pepper. The fungus lives over winter on decayed peppers left in the field, also on the seed from such decayed fruits. It may persist in or on the surface of seed in storage at least 18 months. Histological study of inoculated pepper fruits has shown that the fungus spores produce appressoria on the end of short germ tubes. From these appressoria a thin penetration hypha passes into the host tissues usually within 2 to 4 days, under moist conditions. By this time the epidermal cells near the appressoria are killed. Up to this stage the development in green and ripe fruits appears similar, but in green fruits there is little further development of the fungus. The mycelium develops very slowly in these dead cells until the fruit begins to ripen. The fungus then begins rapid growth and a decayed spot is soon evident. In inoculated ripe fruits spots develop in 6 to 10 days.

Studies on Bordeaux deposition. GEORGE L. HOCKENYOS and GEORGE R. IRWIN.

There is described a laboratory technique of applying a definite amount of spray to selected leaves. This uses the principle of a pendulum shutter cutting off a constant jet of spray. Methods of sampling and analyzing are described whereby both the amount deposited per unit area and the uniformity of deposit are determined. It is shown that the addition of a wetting agent increases the uniformity of deposit but does not decrease the amount deposited per unit area. The amount of deposit is shown to correspond closely to that found by Guba. Dried blood albumin was used in most of the work but comparisons were made with soap and several wetting agents.

The relation of maturity of seed to seedling-blight susceptibility in dent corn. P. E. HOPPE, J. R. HOLBERT, and J. G. DICKSON.

The expression of resistance to seedling blight in corn caused by *Gibberella saubinetii* is affected by many environmental factors. It has been found that the environment during the growth and maturation of the mother plants greatly affects the disease reaction of seedlings of the subsequent crop, when inoculated with *G. saubinetti,* regardless of the genetic potentiality for resistance in the strains. Results from experiments have shown that different degrees of maturity of the seed influence the disease reaction and the yield of the crop. Several hundred self-pollinations in inbred lines were made, the ears were harvested periodically, and the resulting seed was inoculated, planted, and rated for resistance to seedling blight. Results indicated that resistance to seedling blight increased with the maturity of the parent seed. However, the rates of increase in resistance varied at the different stages of maturation of the parent seed. Assuming the disease reaction of the seedling to be an index to the actual degree of maturity of the parent seed, it appeared that some strains, relatively slow in development in the earlier stages, ripened more rapidly and actually matured earlier than others that had previously appeared more mature. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Wisconsin Agricultural Experiment Station, and Funk Bros. Seed Company.)

Avocado diseases in California. WM. T. HORNE.

Avocado production and marketing appear to be established on a permanent basis in California. The plant diseases that have influenced this development up to the

present time have been of a somewhat unusual and special character. *Water injury* or *Melanorhiza* causes death of trees. *Tipburn* is a symptom, usually associated with some phase of the alkali problem. *Chlorosis* and *little leaf* apparently parallel similar diseases of citrus. *Sun blotch* is an infectious chlorosis. An important *canker* of *green stems* has not yet been solved. *Carapace spot* of fruit is due to very early and slight surface injury. *Thompson spot* is a necrosis on the lower end of green fruit, followed by internal changes, which cause loss of the affected fruits; cause not determined. *End spot* is a breakdown of mature or overmature fruit. Spoilage of mature fruit on the tree or of fruit that has been removed and is in process of softening is caused, in approximately the given order with regard to ripeness of fruit by Dothiorella, Colletotrichum, Fusarium, Alternaria, miscellaneous organisms, Rhizopus, and putrefactive bacteria.

Effects of root rot on the physiology of peas. JAMES G. HORSFALL, Z. I. KERTESZ, and E. L. GREEN.

A study was made of the effect of pea root rot (a disease complex caused by a series of fungi) on quality, maturation, and chemical composition of canning peas. Peas from normal and diseased plants were compared by following, during the ripening period, the changes in size distribution, load necessary to crush a single pea, and dry-matter, ash, nitrogen, and crude-fiber content. At first, diseased peas enlarged more rapidly than normal but soon lagged behind and never attained normal size. Since the crushing load for a single pea was higher, size for size, on the same harvest date, for diseased than for normal peas, the quality of the diseased peas was lower. On an equal dry-matter basis, however, crushing load was lower for diseased than for normal peas. Possible reasons: (1) diseased peas dried out before they were filled, thus being smaller than normal; (2) the construction or composition of the dry-matter stuff was weaker; (3) ash and nitrogen contents were always lower. Crude-fiber content, however, seemed to bear no significant relation to healthiness. Growth curtailment and lowered quality in diseased peas seemed to be due to lowered water content and consequent decreased metabolism and translocation.

The fungi present on the surfaces of normal apples and their relation to decay. GLENN A. HUBER.

The surfaces of normal apples obtained from 4 important apple-growing districts of Washington—Kennewick and the Spokane, Yakima, and Wenatchee valleys—were studied to determine the fungi present. One hundred and twenty-four species, or forms, belonging to 29 genera, not including 23 unclassified, non-rot-producing forms, were isolated from the surface of normal apples. Fifty-eight of these species, or forms, were found capable of causing distinct decay when inoculated into sound, ripe Jonathan apples, of which 34 were capable of causing decay when incubated at 0–2° C. Over 20 species belonging to 14 genera were obtained that had not been previously recorded on apples in the State of Washington. The following fungi, found capable of causing decay of apples, are reported for the first time: *Sporormia* sp., *Pyrenochaeta* sp., *Chaetomella* sp., *Aspergillus* sp., *verticillium* sp. (Forms 1, 2, and 3), and *Podosporiella* spp. (Nos. 1 and 2).

Stewart's disease of corn. S. S. IVANOFF.

Studies on the Stewart's disease of corn, caused by *Aplanobacter stewarti*, have shown that the pathogen may enter the roots of corn plants grown in artificially infested soil through wounds produced artificially or through wounds produced by white grubs, the larval stage of *Phyllophaga* sp. The location of the pathogen in the corn tissues

was determined by means of histological and isolation studies. In the leaf tissue the bacteria escaped from the vessels and entered the parenchyma. Their activities within ruptured cells and between cells caused discoloration, plasmolysis, and death. In the kernel the bacteria were observed in vessels and in adjoining cavities of the chalazal region, between the testa and the aleurone layer and between the endosperm cells. In the tassel the bacteria were located in the vessels of rachises, rachillas, glumes, and filaments. The bacteria were isolated from exudate on leaves and the water at the base of unfolding leaves, from the glumes, anthers, and pollen of diseased plants, and from corn stubble overwintered in the field. A selective medium was developed with which the bacteria were recovered from the soil.

Scab and canker found on Citrus in herbaria of England and the United States. ANNA E. JENKINS and H. S. FAWCETT.

Citrus material has been examined at the Herbarium, Royal Botanic Gardens, Kew, and the Department of Botany, British Museum, South Kensington, London, England, and Gray Herbarium and the Arnold Arboretum, Harvard University, Cambridge, Mass., the New York Botanical Garden, New York, N. Y., the Philadelphia Academy of Natural Sciences, Philadelphia, Pa., and the United States National Herbarium, Washington, D. C.

The examinations revealed the occurrence of both Citrus scab and canker at dates and in localities not previously recorded. Scab was found on collections from Japan made in 1862, from India in 1868, and from China and Korea at later dates. Also, what is apparently scab was present on a specimen from Java collected as early as 1840. Canker was found on collections of Citrus recorded from Java as early as 1842 to 1844, from the Philippines in 1903, and from other countries at more recent dates.

The presence of starch near wounds in Burley tobacco plants.—BURT JOHNSON.

In the preparation of concentrated suspensions of the virus of tobacco mosaic it was necessary to measure the strength of the various materials in the most quantitative manner possible. Leaves were punctured with virus-bearing pins, according to a method described by Holmes, and then by the iodine reaction, the amounts of virus present were determined by the percentages of wounds having excessive amounts of starch around them. When Burley tobacco was used as the host plant there was no constant diminution in the number of local lesions with increase in dilution of the virus. Juice from healthy plants used as inoculum gave essentially the same results as juice from diseased plants. Different chemical treatments of the virus did not appreciably affect the percentages of lesions found. Water inoculated into the plants or wounds of several kinds gave numbers of lesions about equal to those found when virus was inoculated into the plants. If the leaves had little or no starch in them to begin with, lesions, regardless of the inoculum used, were few in numbers and very indistinct. Other plants were tested in a similar manner and like results were obtained according to whether or not the leaf was well supplied with starch.

Verticillium wilt of chrysanthemums. LEON K. JONES and GLENN A. HUBER.

Verticillium wilt is prevalent in greenhouses in Washington State and 100 per cent damage to the crop has been noted with certain varieties. The following varieties have shown marked susceptibility to the disease: Adrian's Pride; Chadwick; Distinction; Dr. Enguehard; Favorite; Gladys Pearson; Golden Measure; Helen Frick; Justrite; Mrs. Simpson; October Rose; Mistletoe; Quaker Maid; Rose Glory; Rose Royal; Seidewitz; Silver Sheen; Whittier; and Wolf's Pink. The following varieties appear to be highly resistant to the disease: December Beauty; December Glory; Early Frost; Friendly

Rival; Gold Lode; Golden Queen; Honey Dew; Improved Golden Glow; Indianola; Lustre; Monument; Wm. Turner; W. H. Waite.

Preliminary investigations tend to show that the organism is carried in the cuttings taken from diseased plants of the more susceptible varieties. Control recommendations should emphasize the use of cuttings from healthy plants as well as soil sterilization.

Bactericides in relation to Bacillus amylovorus and fire-blight control. G. W. KEITT, LUTHER SHAW, and A. J. RIKER.

Relative toxicity of various chemical compounds and spray preparations to *Bacillus amylovorus* was studied, in which a modified Rideal-Walker technique was used. Silver and mercury salts showed outstanding toxicity; copper and cadmium salts, a low intermediate degree; nickel and iron salts, relatively low toxicity. Compounds of mercury varied much in relative toxicity, certain organic compounds and mercuric chloride being most toxic. Bordeaux mixture, zinc sulphate-lime mixture, and lime, respectively, killed the bacteria at concentrations lower than commonly used in sprays.

Spray programs were applied to large apple trees in which suitably located blossom clusters had been inoculated by a standardized procedure to furnish an abundant natural fire-blight inoculum. Abundant blossom blight developed on nonsprayed inoculated trees, the chief natural infection period coming at late full bloom, when there was a 2-day-rain period. The spray application made at full bloom seemed to be the most effective. Bordeaux mixtures, 3–6–50 and 1–3–50, excellently controlled blossom blight. However, the stronger Bordeaux severely russeted the fruit. In a single trial lime, 3–50, gave very promising results. Various mercurial sprays were less effective, reducing blossom blight by approximately $\frac{1}{2}$ to $\frac{3}{4}$. Recommendations are withheld pending further work.

Fasciation in red pine. RAYMOND KIENHOLZ.

A red pine, *Pinus resinosa*, about 20 feet high and 18 years old, growing in a plantation of the Yale Forest at Keene, N. H., was found to exhibit extensive fasciation of the leader and upper laterals during the past 4 years of its growth. Because of the rarity of fasciation in conifers in general and pine in particular, it is of interest. The upper part of the tree is bushy, the fasciated branches being bunched and bearing great masses of needles. The leader seems particularly subject to fasciation and, when fasciated, a lateral bends upright to take its place, only to become fasciated in turn.

Moisture of shelled corn in relation to fungus growth. BENJAMIN KOEHLER.

Shelled yellow-dent corn was placed over calcium chloride solutions of different concentrations in closed vessels, some series aerated, others not. Twelve different humidities were provided in which the corn moistures ranged from 9.6 to 32.0 per cent (wet basis). In some series internally infected corn was surface sterilized and maintained free from contamination so as to observe better the effect of certain infections. All series were kept for 2 months at room temperature before final observations were made. No fungus growth occurred at 14 or less per cent moisture in these experiments. Aspergillus growth was found at 14.9 and above. "Blue eye," a Penicillium growth over the germ but beneath the pericarp, occurred frequently at 19 to 21 per cent. *Diplodia zeae* became active at 21.2 and *Fusarium moniliforme*, *Gibberella saubinetii*, and *Basisporium gallarum* at 22 to 23 per cent. Diplodia grew more extensively and was more destructive at marginal and higher moistures than the 3 last-named fungi. Aeration had little effect on determining the minimum moisture range of the fungi observed, but in some cases lack of aeration checked their growth.

Local lesions in Aucuba mosaic of tomato. L. O. KUNKEL.

Studies on Aucuba or yellow mosaic of tomato obtained from England show that the virus of this disease produces necrotic local lesions in leaves of the following species and varieties of Nicotiana: *N. acuminata, N. alata, N. glutinosa, N. langsdorffii, N. rusbyi, N. rustica, N. suaveolens, N. sylvestris, N. tabacum* var. Adcock, *N. tabacum* var. Burley, and *N. tabacum* var. Little Oronoca. It produces chlorotic local lesions in the following species and varieties: *N. paniculata, N. tabacum* var. Connecticut Seed Leaf, *N. tabacum* var. Macrophylla, and *N. tabacum* var. Purpurea. It also produces chlorotic local lesions in leaves of the following varieties of tomato: Beef Steak, Early Freedom, Globe, Golden Queen, John Baer, June Pink, Matchless, Red Pear, Red Plum, Red River, Stirling Castle, and Yellow Cherry. The production of local lesions is associated with multiplication of the virus at the point of inoculation. Aucuba mosaic differs from the ordinary tobacco mosaic in its capacity to produce local lesions in the tomato and in certain species and varieties of Nicotiana.

Factors affecting infection and decay of sweet potatoes. J. I. LAURITZEN.

Host response of Citrullus vulgaris to Colletotrichum lagenarium. DUKE V. LAYTON.

Colletotrichum lagenarium produced maximum infection on *Citrullus vulgaris* in the greenhouse at 20 to 28° C. and a relative humidity of 93 per cent. Less infection was secured at 17° and none at 15° C. with nearly the same humidity (90 per cent).

The reaction to the anthracnose organism of 43 varieties from 9 countries, 416 mass selections from the varieties Kleckley Sweet, Conqueror, and Halbert Honey, and 46 hybrids was determined by indexing in the greenhouse. Of these, 116 were given further trial in the field. The citrons (particularly Majorta, an African forage melon) showed a high degree of resistance, but most of the oriental varieties were markedly susceptible. The F_2 and F_3 generations of 5 citron-watermelon hybrids were appreciably less susceptible than commercial watermelons. Several inbred segregates from chance hybrids of Conqueror have shown marked resistance to *Colletotrichum lagenarium.* These are selections from Iowa Belle (Q21), Iowa King (Q23), and Q20, which are also edible and wilt resistant.

New parasites of cereal rusts. M. N. LEVINE, A. A. GRANOVSKY, and J. G. LEACH.

In the course of experiments dealing with the dissemination of rust spores by the garden slug, *Agriolimax agrestis,* wheat seedlings in the greenhouse were inoculated with urediniospores of *Puccinia graminis tritici* that had passed through the intestinal tract of slugs. Neither the virulence nor the specialization of the physiologic forms tested was changed. However, many of the resulting pustules were parasitized by a fungus and a bacterium. The effect was most pronounced on rusted seedlings, but the rust on adult plants also was severely affected. The organisms were isolated in pure culture and the action of each has been studied separately. Neither the bacterium nor the fungus appears to be pathogenic to healthy plants. High relative humidity is a prerequisite for the growth of the fungus parasites; whereas the bacterium thrives well under normal climatic conditions, whether in the field or in the greenhouse. All of the cereal rusts are apparently susceptible. The identity of the parasites has not yet been determined.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Division of Entomology and Economic Zoology and the Division of Plant Pathology and Botany, University of Minnesota.)

Present status of the Dutch elm disease. O. N. LIMING.

In the summer of 1931 4 trees affected with the Dutch elm disease were found in Cleveland, Ohio, thus making a total of 8 cases in America. The original source of the disease in this country has not been found. About 600 diseased elm trees were examined. Several other elm diseases were encountered in the investigation. In inoculation experiments, infection of American elms was secured by several hypodermic injections at one level and by incision wounds. Chinese and Siberian elms, inoculated with *Graphium ulmi*, showed only internal symptoms of the disease.

Graphium ulmi survived the winter in artificially inoculated twigs placed outdoors under a bell jar. In a branch in which only the 1929 ring was discolored, there was no evidence of the fungus passing outward to the next year's ring. The fungus in a diseased tree trunk, placed on moist soil in the greenhouse, produced coremia on the bark and in borer tunnels. Branches from a diseased tree became sterile after 2 months in a dry laboratory. The fungus has its highest growth rate between pH 5.0 and 6.0 and at 22° C.

(Cooperative investigation between Division of Forest Pathology, Bureau of Plant Industry, and Ohio Agricultural Experiment Station.)

Progress in the control of stain and mold fungi that discolor stored lumber and logs. R. M. LINDGREN, T. C. SCHEFFER, and A. D. CHAPMAN.

Sapwood of both logs and lumber is liable to stains (caused by species of Ceratostomella and imperfect fungi with dark hyphae) and superficial molds (mainly Moniliaceae). These have been a considerable commercial handicap in the South, even where the best handling methods were employed. Following extensive small-scale elimination trials with fungicides, commercial tests were conducted in 1930 and 1931 at mills in several States. In most places satisfactory control has been secured on hardwoods—gum (Liquidambar), poplar (Liriodendron) and oak—with 5 per cent borax solution and on both pine and hardwoods with 0.01 per cent ethyl mercuric chloride; good results have also been secured with sodium salts of tetrachlorphenol or orthophenylphenol, or mixtures of the two, in concentrations of approximately 0.4 to 0.5 per cent. At the larger mills lumber is carried by the regular conveyors through a vat containing the cold solution; costs of the fungicides approximate 12 cents per M board feet, or only about 3/5 cent per hundred square feet of surface protected. Many mills have now adopted one of the new fungicidal treatments. In small-scale tests 0.4 per cent sodium 2-chlororthophenylphenolate appeared promising on pine and 3.6 per cent boric acid was effective on hardwoods.

Some of the treatments have been found effective in small-scale tests as a spray in preventing the deterioration caused by stain and decay fungi in logs stored during the winter months.

Etiological concepts of plant pathology. GEORGE K. K. LINK.

Engrossed in control problems, dominated by germists, and neglectful of theoretical development of its etiological subject matter, phytopathology is considered "applied mycology" and has failed to gain recognition as a botanical science commensurate with that afforded physiology and genetics. As a pure science it is not motivated by practical considerations, includes all pathic events, and has more in common with physiology and genetics than with mycology.

Adequate development of etiological pathology involves recognition that the organization of the pathic organism plays a pathogenic rôle in practically every pathic event. Further analysis of the antecedents of a pathic event occurring at a given instant and

place involves consideration of (1) the genetic constitution of the place; (2) the past history of the place; (3) the correlative influences acting on the place; and (4) the immediate influence of the external environment (including pathogenic organisms). It involves use of the concepts and data of genetics, developmental physiology, and biochemistry as much as those of ecology (host-parasite relations and relations of nonliving environment factors to pathic and pathogeneic organisms).

This approach does not ignore "hereditary" and noninfectious diseases and integrates susceptibility, infectivity, and virulence phenomena in a biologically-sound etiological system.

The precipitin-ring test applied to fungi. GEORGE K. K. LINK, ADELINE DES. LINK, GEORGE L. CROSS, and HAZEL W. WILCOX.

A procedure has been devised that permits application of the precipitin-ring test in the study of proteins of fungi. Fungus mats grown in protein-free synthetic medium, upon proper washing, drying, grinding, and extraction with fat solvents, yield highly antigenic extracts in 0.85 per cent NaCl solution. The nitrogen content of these extracts has been determined by micro-Kjeldahl digestion and their protein content calculated. The extracts remain clear, do not cloud, nor give nonspecific precipitates with normal serums, but give definite and graded precipitin rings when progressively higher dilutions in 0.85 per cent NaCl are layered on homologous and heterologous antiserums.

Details of procedure have been determined for each step in production of antiserums (preparation and dosage of antigens, interval, frequency, and method of injection into rabbits), and in execution of tests (relative merits of various mediums of dilution and of types of layering; rôle of pH of antiserums and test antigens and of time and temperature factors).

The method has been applied to 23 species and varieties of Fusarium and to 1 species, each, of Cylindrocarpon, Gibberella, Hypomyces, Neurospora, Ramularia, and Sclerotinia. The precipitin-ring test gives promise of applicability in mycological and mycophytopathological studies.

(Aided in part, since 1929, by a grant to the University of Chicago from the Rockefeller Foundation.)

Parasitism of Diplodia zeae on the crown of the corn plant. GEORGE L. MCNEW.

On land previously cropped to corn, plants from nearly disease-free seed had 12 per cent of crown infection with *Diplodia zeae* Lev., and plants from treated Diplodia-infected seed had 18 per cent. One hundred per cent of the plants grown in steamed soil infested during the seedling stage with pure cultures of *D. zeae* became infected. Less infection resulted and progress in the crown was retarded when the soil was infested later in the growing season. At maturity 80 per cent of the plants showing severe infection of the crown were invaded to the second internode. Under similar conditions, except that the inoculum was omitted, the same symptoms were produced on plants grown from seed naturally infected with *D. zeae*. A series of plants showing 81 per cent mesocotyl infection, but escaping seedling blight, showed 80 per cent crown infection when the crop was mature. The pathogen invades the crown either from the soil or from infected seed.

An experiment on the influence of soil moisture on crown infection indicated that root reduction was greatest in compost soil at 45 per cent of the water-holding capacity (42 per cent). In a second experiment 80 per cent infection developed at 45 per cent soil moisture, which was higher than occurred at either 30 or 85 per cent soil moistures.

Cyclamen stunt. L. M. MASSEY and PAUL E. TILFORD.

This disease was observed in New York in 1926 and in Ohio in 1929 and is known to occur in Pennsylvania, New Jersey, and California. Affected plants are conspicuously stunted but not quickly killed. Leaves are small and may be yellow. The petioles and peduncles are shorter than normal and the flowers characteristically open below the leaves. Necrotic areas, reddish brown in color, are found in the tissues of the corm. They are typically confined to the crown of the corm but may extend throughout—even for short distances into the petioles, peduncles, and the larger roots.

Cyclamen stunt is caused by an undescribed species of *Cladosporium*, for which the name **C. cyclaminis** n. sp. is proposed. Successful infection experiments have been conducted both with spores and mycelium. The fungus grows slowly in culture, producing on potato-dextrose agar a raised or stroma-like greenish black thallus commonly covered with a grayish white aerial mycelium. Spores are formed in culture in from 4 to 7 days, acrogenously, singly or in short chains, on short rarely-branched conidiophores. They are hyaline, becoming somewhat brown with age, typically 0- to 1-septate, and average 17.75 x 4.3 μ.

Control of basal rot of narcissus. L. E. MILES.

In 1928, 1929, and 1930 experiments were conducted on control of a basal rot of narcissus bulbs caused by a species of Fusarium. Preliminary tests showed conclusively that the disease is disseminated by the hot-water treatment as required by the Federal Quarantine Act against the bulb eelworm and bulb flies. Mercuric chloride, formaldehyde, Semesan, and Ceresan used in conjunction with the hot-water bath prevented this dissemination and gave varying degrees of control of the rot. Ceresan, at a concentration of 1: 200, gave 99 per cent healthy bulbs; formaldehyde, concentration 1: 240, 88 per cent healthy; with mercuric chloride and Semesan intermediate as compared with 82 per cent healthy in the dry, untreated checks and 59 per cent healthy in the bulbs undergoing the unmodified, hot-water bath.

In treatment of bulbs immediately after or without hot-water treatment Calogren 1: 20, used as a dip, gave much better control of disease than any other chemical tested. All treatments yielded large increases in yield over the untreated bulbs. This increase was directly proportional to the degree of disease control attained. Dust treatments proved less efficient than did the liquid dip and soak treatments.

Verticillium wilt of cotton in Mississippi. L. E. MILES and T. D. PERSONS.

In the fall of 1929 a wilt or hadromycosis of cotton, caused by a species of Verticillium, tentatively determined as *V. albo-atrum* was discovered on experimental plots at the Delta Branch Experiment Station at Stoneville, Miss. In the fall of 1930 and the present year a survey was made by means of actual examination of fields and by soliciting specimens from county agents, State Plant Board inspectors, and others to secure an idea of the comparative distribution and prevalence of the trouble as compared to Fusarium wilt, from which it is, with our present knowledge, indistinguishable except by cultural methods.

All specimens collected or received were cultured and the presence or absence of *Fusarium vasinfectum* or Verticillium recorded. The specimens cultured represent collections from 30 of the 82 counties in the State, widely scattered on all types of soil. One-sixth of the collections have yielded the Verticillium fungus. One-fifth of those showing Verticillium have given cultures of Fusarium, also. All others have shown only Fusarium. Verticillium wilt has been found in 8 counties, namely, Tallahatchie, Bolivar, Sunflower, Coahoma, De Soto, Washington, Tunica, and Leake. All, with the exception of Leake, are in the Delta section.

The genetics of Ustilago zeae. M. B. MOORE.

A study was made of the gametic (F_1) segregates from crosses between 1 monosporidial line and each of 2 others that had contrasting cultural characters. All of the F_1 lines isolated from the 2 crosses fell into 17 cultural groups, 49 from 1 cross falling into 12 and 38 from the other into 13 groups. A few F_1 lines were almost identical with one or another of the parental lines. All characters studied, including sex, apparently were governed by multiple factors. Segregation of factors for cultural characters occurred in the second division of the fusion nucleus or began in the first and was completed in the second. Occasionally segregation may have occurred in still later divisions. Three different combinations of the F_1 lines from a single chlamydospore differed greatly in their ability to form chlamydospores on corn plants, even though all produced abundant galls consisting of fungus mycelium and hypertrophied host cells. The number of spores formed varied with the variety or line of corn inoculated. One combination, although producing numerous large galls, formed chlamydospores very sparingly and only in occasional galls.

Sporulation of 5 species of Cercospora in pure culture. CLATUS M. NAGEL and S. M. DIETZ.

The failure of many species of Cercospora to sporulate abundantly in pure culture has been one of the limiting factors in studying the biology, classification, and cross inoculation of the species of this genus. During the past 9 months transfers from stock cultures of *Cercospora beticola, C. dubia, C. davisii,* and *C. zebrina* have been grown on 30 different media. During this time 40 successive transfers were searched for conidia as soon as they showed vigorous growth, and no conidia were found. During the summer of 1931 Cercospora was isolated at Kanawha, Iowa, from the following hosts: *Beta vulgaris, Setaria glauca, Melilotus officinalis, Chenopodium album,* and *Physalis pubescens.* Cultures of all 5 of these species on potato-glucose agar sporulated 48 hours after the original isolation. Ten successive transfers with conidia have been made from each of these cultures at 5-day intervals held at 24–27° C. In all of the cultures abundant conidial production occurred over the entire surface of the culture. Isolations made from *B. vulgaris* sporulated more abundantly on sugar-beet-leaf agar than on potato-glucose agar. Stock cultures of *Cercospora beticola, C. dubia, C. davisii,* and *C. zebrina* produced no conidia when exposed for different lengths of time to ultraviolet irradiation.

A greenhouse tomato hybrid somewhat resistant to Cladosporium leaf mold. A. G. NEWHALL.

Out of 4 English tomato varieties, procured from Dr. Bewley in 1928, two possess considerable resistance. Infection can occur but is delayed. The plants seem to be hypersensitive, so that very little sporulation takes place. Secondary infections are thus much delayed. Since none of the fruits of the English varieties Satisfaction and Main Crop seem to have the size and flesh characters demanded by the American markets, they have been crossed with Bonny Best and a pink Marglobe (Marhio). In the F_1 generation susceptibility was dominant. Seedlings of the F_2 generation were inoculated and the susceptible ones discarded. Some of the resistant ones were grown in ground beds until their 8th cluster had matured. Yields varied between 5 and 12 pounds per plant. Final resistance also varied somewhat as the plants aged.

Two commercial growers have been further testing the F_3, F_4, and F_5 generations. The aim has been to develop a midsize red tomato. Complete resistance has not been obtained, but a strain has been selected that is slower in developing the disease under

greenhouse conditions in New York State. Back crosses have been made to increase the size of fruit.

Root and storage rot of celery caused by Phoma apiicola. A. G. NEWHALL.

Late celery from certain muck lands in New York develops a soft, dark, greenish butt rot, after a few weeks in commercial cold storage (0° C.), rendering it unfit for market. This rot, caused by *Phoma apiicola,* is easily distinguished by its greenish color and less watery consistency from that caused by *Sclerotinia* sp. There is no evidence of disease at the time of harvesting the crop and placing it in cold storage in October. No important evidence of resistance to the storage rot has been found among 20 varieties tested, all showing from 18 to 90 per cent decay within 9 weeks.

By growing plants in artificially inoculated muck soil held at different temperatures (14°, 19°, 29° C. $\pm$ 1½°) and different moistures (dry, medium, and wet) the root rot has been studied under a variety of conditions Soil moisture had little influence. The fungus grows slowly and has a low optimum (18° C.) and a very low minimum temperature in pure culture. Isolations have been made from the roots of plants showing that there is very little evidence of root rot. On placing these plants in cold storage, however, at a high humidity, they decay almost completely in 7 weeks.

Variability of Venturia inaequalis in cultural characters and host relations. D. H. PALMITER.

Monoconidial cultures of *Venturia inaequalis,* isolated from 14 apple varieties from Wisconsin, Oregon, Michigan, and New York, showed cultural differences not correlated with the locality or variety from which they were isolated. Differences were of the following types: amount of aerial mycelium, margin of colony, rate of growth, and abundance of conidia or ascocarps produced in culture. In greenhouse inoculations infection occurred only on Malus species. Amelanchier, Aronia, Cotoneaster, Crataegus, and Sorbus species were not infected. *Malus toringoides, M. sieboldii, M. ioensis, M. sargentii, M. niedzwetskyana, M. baccata, M. coronaria, M. tschonoskii,* and *M. robusta* were each infected by one or more of the cultures. *Malus floribunda, M. angustifolia, M. arnoldiana,* and *M. theifera* were not infected. Under the conditions of the experiments Yellow Transparent was infected by 5 cultures and resistant to 4; McIntosh, infected by 2 cultures and resistant to 3; Dudley and Missouri Pippin, infected by 3 and resistant to 2; Hubbardston Nonsuch, infected only slightly by 3 and resistant to 2 of the cultures. The other 15 apple varieties inoculated were infected by all of the cultures.

Infection studies with Mycosphaerella fragariae and Diplocarpon earliana. A. G. PLAKIDAS.

From a large number of tests it has been shown that infection on strawberry leaves with *Mycosphaerella fragariae* and *Diplocarpon earliana* takes place primarily, if not exclusively, through the epidermis on the lower side of the leaf. Inoculations were made by smearing spore suspensions on either the upper or lower leaf surfaces with a camel's-hair brush. In the case of Mycosphaerella ingress is stomatal, and there appears to be a correlation between the amount of infection and the number of stomata on the leaf surfaces. With the variety Klondike the average number of stomata on the upper and lower leaf surfaces was found to be 3.16 and 147.77 per square millimeter, respectively. The mode of ingress in the case of Diplocarpon has not been determined definitely, but it does not appear to be stomatal. The fact that infection takes place primarily through the lower surface of the leaf may possibly be explained by the difference in the thickness of the cuticle and epidermal layers on the upper and lower surfaces, respectively.

The "June yellows" of strawberries. A. G. PLAKIDAS.

The name "June yellows" is suggested for a disease or a peculiar yellow condition of the strawberry, which occurs in the Northeastern States and in Canada. This name is applicable because the symptoms are more pronounced in early summer. From certain of its symptoms, including the yellowing, mottling or marbling of foliage, stunted growth, and vegetative transmission from a mother plant to all its daughters through runners, the June yellows resembles diseases of the virus type. All attempts, however, to transmit it by juice inoculation and leaf mutilation, by the use of soil, by insects [white flies, leaf hoppers, and aphids—*Aphis forbesi* and *Capitophorus fragaefolii* (?)], and by bridge grafting have thus far failed. The bridge-grafting technique has been developed so that a complete union has been obtained with 75 per cent of the grafts. A certain percentage of plants growing from seeds from affected plants finally show the yellows symptoms, though these are not evident during the first year. The disease has been found in Massachusetts, Maryland, New York, New Jersey, Michigan, Ohio, Wisconsin, and Canada on one or more of the following varieties: Eaton, Beaver, Howard 17, Mastodon, Peerless, Van Dyke, Vineland seedling 2,532,192, Superb, and 23 different seedlings of the U. S. Department of Agriculture.

Reaction of tomatoes to mosaic. R. H. PORTER.

A study was made with 27 pure lines of tomatoes, each representing a variety or type. From 5 to 8 plants of each line were inoculated in 2 sets with tomato-mosaic virus. All of the plants in lines 11 and 13 developed symptoms in 10 and 11 days, respectively. In one line, 838–4, 2 plants out of 8 showed symptoms 11 days after inoculation and 4 more were infected the following day. The 2 remaining plants developed symptoms in 20 days. Five inoculated plants of line 398–4 developed no symptoms, but the virus was present, as was proved by transfers back to highly susceptible plants. Repeated trials with this line gave the same results. Fifty-nine plants of an F_2 generation (line 574–4), in which line 398–4 was one of the parents, were inoculated with tomato-mosaic virus and 12 remained free of symptoms.

Plants of 20 pure lines were inoculated with the virus of yellow-tomato mosaic (probably identical with Johnson's tobacco virus 6) and all of the plants in 3 lines remained free of outward symptoms.

Some diseases of wild potatoes in Mexico. DONALD REDDICK.

Blight, caused by *Phytophthora infestans*, was found on *Solanum verrucosum* from the State of Morelos. Rust, caused by *Puccinia pittieriana*, occurs abundantly on *S. demissum* at El Desierto in the Federal District but was not encountered elsewhere in the *tierra fria*. Spot, a disease of unknown cause, is generally prevalent on several tuber-bearing species. Viroses and tuber diseases either were not encountered or else were not recognized on wild plants.

Basisporium dry rot of corn. CHAS. S. REDDY.

Injury by *Basisporium gallarum* is associated with cessation of translocation within the corn plant. It occurs at the time of germination, after normal maturity, and when plants die prematurely from cold or other causes. Basisporium-infected seed dies within a few days after it is planted in soils slightly below the temperature range for germination. The organism kills the germ before active translocation sets in. Therefore, poor field stands are correlated with cold soil temperatures at time of planting. Seed treatments are most beneficial under conditions of greatest injury by the organisms, such, for example, as cold soil at time of germination.

Seed-corn strains that germinate readily at low temperatures (below 11° C.) are injured little by *Basisporium gallarum*. Experiments on artificial inoculation of corn ears with *B. gallarum* indicated that natural inoculum was widespread at the time and that the number of infected ears was not significantly increased by artificial wound inoculations. With or without artificial inoculation, susceptible ears became infected and the resistant ears did not. The resistant ears are those having high hydrogen-ion concentration in the cobs. Cob reaction is an inherited character and promises a means of more easily breeding strains of corn resistant to Basisporium ear rot.

Clitocybe mushroom root rot—a new disease of citrus trees. ARTHUR S. RHOADS.

Approximately 150 citrus trees in Florida, comprising grapefruit, oranges, and tangerines on rough lemon rootstock, have been found attacked by root rot caused by *Clitocybe tabescens*. This disease appears to be of long-standing occurrence in Florida citrus groves but has not been differentiated heretofore from foot rot.

Clitocybe mushroom root rot resembles that caused by the honey agaric or oak-root fungus (*Armillaria mellea*) in habit of growth, production of rhizomorphs, appearance of the mushroom-like fruiting bodies, and preponderance on land where oak trees have occurred.

The discovery of mushrooms at the bases of the trunks led to the location of many attacked trees in which the healthy appearance of the tops did not suggest the occurrence of root rot. In the large number of attacked trees in which the root systems were investigated a good proportion of the lateral roots, and usually the taproot also, was found to be invaded by Clitocybe and often well rotted before the tops showed any evidence of decline. In most of the cases oak, and occasionally other hardwood roots, were found, which were extensively invaded by the mycelium of this fungus, these often being in contact with and serving to transmit the disease to the citrus roots.

Clitocybe mushroom root rot—a new disease of bananas. ARTHUR S. RHOADS.

Mushroom root rot, caused by *Clitocybe tabescens*, has been found attacking and killing banana plants at two localities in Florida. At Artesia, where this disease was attacking a considerable number of trees and other woody plants on hammock land that supported a heavy growth of oak and other hardwood trees prior to clearing, a number of clumps, each, of Dwarf Cavendish, Hart's Choice or Lady Finger, and Orinoco or Horse bananas were found attacked. An additional record of the occurrence of this disease of bananas was afforded by a specimen received from Nocatee.

The bases of the attacked plants were involved in a watery, brownish discoloration, with sheets of mycelium and a luxuriant development of rhizomorphs between the leaf-stalk bases, which comprise the trunk, and within the septate tissues of the same. The rhizomes of the attacked plants were permeated by numerous mycelial sheets. The development of the mycelium through the rhizomes serves to transmit the disease to other plants in the clump and eventually the entire clump is killed.

Pure cultures of the fungus associated with the disease have been grown and carried to fruition in comparison with cultures of *Clitocybe tabescens* isolated from several other plants killed by this mushroom root-rot fungus.

Two forms of fire blight and a new related disease. H. R. ROSEN.

On Jonathan apple trees 2 different forms of blight have been distinguished with reference to different sources of inoculum. One form, early or principal epidemic of blossom blight, bears no discernible relationship to hold-over blight, and the second or later form, exclusive twig blight, is directly related to hibernated cankers and blighted

twigs present on the trees. The first may appear in different degrees of severity regardless of the presence or absence of blight the preceding year; the second is intimately associated with last year's blight.

Another bacterial disease of pomes, hitherto unreported, the symptoms of which simulate fire blight to a considerable degree, exists in America. It consists of a blossom blight, a fruit rot, and a leaf spot, but has not yet been found to involve a twig or limb blighting under natural conditions, though twig blight is readily induced with pure-culture inoculations. The pathogen is closely related to *Bacillus amylovorus* but shows numerous distinct cultural, physiological, and pathogenic properties. It belongs to the group of bacteria all of which have been noted in the literature as being akin to *B. amylovorus*, consisting of *B. barkeri* Berridge, *B. nectarophilum* Doidge, and *Pseudomonas prunicola* Wormald.

Physiologic strains of Fusarium niveum. BAILEY SLEETH.

The failure of the Conqueror watermelon to prove resistant to watermelon wilt in the different melon-growing sections has suggested the possibility that there are strains of the wilt organism with various degrees of pathogenicity. Cultures of *Fusarium niveum* were secured from Texas, South Carolina, North Carolina, Iowa, and West Virginia. During 1930–31, greenhouse experiments have been in progress to determine the relative degree of pathogenicity of the various strains of the wilt fungus to certain varieties of watermelons. The ability of the fungus to produce wilt when tested against 6 varieties of watermelons is indicated by the percentage of plants wilted. When tested on 6 varieties, strain 3 produced an average of 90–95 per cent wilting; strains 8 and 6, 85–90 per cent; strains 4 and 1, 80–85 per cent; strain 2, 70–75 per cent; strain 7, 65–70 per cent; strain 5, 45–50 per cent; and strain 9, 20–25 per cent.

The amount of wilting produced by a given strain of *Fusarium niveum* is not always uniform when tested against different varieties of melons. Twenty-three strains have been isolated, which exhibit culture characteristics indicating the possibility of as many physiologic forms.

Diplodia stalk- and ear-rot studies of dent corn. A. L. SMITH and J. R. HOLBERT.

The reaction of numerous inbred and crossbred strains of yellow dent corn to infection by *Diplodia zeae* was determined following hypodermic injections of a spore suspension into internodes of stalks and shanks. Inoculations were made late in August. Extent of spread of infection, including ear rotting, was noted early in October. Significant correlations were found between extent of spread in stalks and extent of ear rot from shank inoculations. The coefficients of correlation between the extents of infection in cortical tissues and pith tissues following hypodermic inoculations and percentages of Diplodia-rotted ears at harvest in the same crossbred strains grown under field conditions and exposed to natural inoculation were $+.84 \pm .05$ and $+.73 \pm .09$, respectively. The results suggest that stalk inoculations with *D. zeae* may be useful in determining relative resistance of stalks and ears of inbred and crossbred strains to Diplodia. Stalk tissues of strains easily injured by exposure to artificial cold in field refrigeration chambers were more quickly invaded by Diplodia than were comparable tissues of strains possessing greater cold resistance. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Wisconsin Agricultural Experiment Station, and Funk Bros. Seed Company.)

Seed transmission of Fusarium wilt of pea. W. C. SNYDER.

Seed transmission of pea wilt (*Fusarium orthoceras*) has been demonstrated in greenhouse trials. Untreated virgin soil from a source previously shown to be free of

the wilt organism was planted consecutively to 5 series of seed of which the first 4, totaling some 8,000, were harvested from wilt-infected plants and the 5th from healthy plants.

During the run of the 4th series, 4 plants in different parts of the seed bed wilted, and the organism was isolated. Additional wilting occurred in the 5th series, indicating the fungus had become established in the soil. The incidence of seed transmission is apparently rare. It seems clear, however, that the fungus is occasionally carried on or in the seed. This serves to explain some observed spread of the disease into widely separated areas.

Isolations of the causal organism from various parts of the United States have not revealed, so far, any differences in their pathogenicity upon susceptible varieties of pea nor in their lack of ability to cause the disease upon resistant varieties, even though considerable variation in cultural characters between isolations from within a given locality, as well as between those from different regions, has been obtained.

Distribution of physiologic forms of Puccinia graminis tritici in the United States and Mexico in relation to rust epidemiology. E. C. STAKMAN, LEE HINES, HARRY G. UKKELBERG, and WALLACE BUTLER.

From 1929 to 1931, inclusive, 27 physiologic forms of *Puccinia graminis tritici* have been identified from about 1,650 collections made in the United States and Mexico. Forms 11, 21, 36, 38, and 49 were by far the most prevalent (averaging almost 90 per cent of all collections) in each of the 3 years, although the order of prevalence of the individual forms varied with the year. Forms 49 and 38 were abundant in Mexico, the latter, which is only weakly pathogenic to the hard-red-spring wheats, constituting about 65 per cent of all collections. Forms 11, 21, and 36, on the other hand, were found very rarely in Mexico. All 5 forms were fairly prevalent in the spring in Texas, where some of them apparently overwintered in the uredinial stage. Results of spore-trap exposures and observations on the northward spread of rust indicate that urediniospores of these forms were carried from Texas northward by high winds. There is abundant evidence also that much inoculum came from barberries in the Northern States. (Cooperative investigations between the Divisions of Barberry Eradication and Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Physiologic forms of Puccinia graminis produced on barberries in nature. E. C. STAKMAN, LEE HINES, RALPH U. COTTER, and M. N. LEVINE.

Many new physiologic forms of *Puccinia graminis* have been produced experimentally by hybridization between existing forms, and many segregates from certain "selfed" heterozygous forms have been isolated from aecia. Presumably, hybridization on barberries also occurs in nature A survey, therefore, has been made of varieties and physiologic forms occurring on barberries and near-by gramineous hosts with primary infection. All North American races or varieties of *P. graminis*, except *P. graminis phleipratensis* develop readily on barberries. The following isolations have been made: 7 forms of *P. graminis secalis;* 17 of *P. graminis tritici*, of which 4 were previously unknown, although 1 was subsequently found on wheat; and 3 forms of *P. graminis avenae*, 1 of which, form 8, has been found only on oats near barberry. Evidently, new physiologic forms are being produced on barberries in nature, and, as mutation in pathogenicity of *P. graminis* seems rare, barberry apparently is primarily responsible for the origin of new forms. (Cooperative investigations between the Divisions of Cereal Crops and Diseases and Barberry Eradication, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Bacterial stalk rot of sweet corn. A. R. STANLEY and C. R. ORTON.

A bacterial stalk rot of corn appeared epiphytotically in the Ohio Valley at Lakin, West Virginia, in 1930 and 1931. The pathogen was isolated from the following varieties in 1930: Aviator, Golden Acre, Golden Sunshine, Golden Coin, Golden Bantam, 60-Day Make Good, and Premo Extra Early, and in 1931 from Alpha, Aviator, Bantam Delicious, Earliest of All, Early Market, Gills' Early Market, Golden Bantam, Golden Bantam (14-row), Golden Bantam (15 x 16 hybrid), Golden Coin, Golden Crosby, Golden 60-Day Sweet, Golden Early Market, Golden Gem, Golden Rod, Golden Sunshine, Mayflower Extra Early, New Extra Early Bantam, Premo, Red-Leaved Early Bantam, Surprise, Burpee, Whipple's Early White, and Early Yellow. Artificial inoculations have been successful when the suscept is grown under sterile conditions on solidified Pfeffer's medium in 2 x 16 inch test tubes. Needle-prick inoculations on the stalk of young corn plants result in the parenchyma's being rapidly invaded, the tissues' turning watery brown and soft, and the stalks crumpling. The numerous strains are motile in broth cultures, produce H_2S when grown on lead-acetate agar, and do not produce indol in tryptophane broth. The organism has been identified as *Bacterium dissolvens* and confirmed as such by Rosen. It shows a striking similarity to *Bacillus carotovorus.*

Sulphur barriers and graminaceous crop barriers to prevent spread of Phymatotrichum root rot. J. J. TAUBENHAUS and WALTER N. EZEKIEL.

Two types of barriers have been tested as possible means of preventing spread of Phymatotrichum root rot. Acid barriers were prepared by mixing 2 or 4 per cent of sulphur with the soil. These barriers were 4 to 6 inches wide and reached to the bottoms of containers or 4½ feet deep in field plats. Cotton was planted on each side of the barriers; those plants on one side only were inoculated with Phymatotrichum. The inoculated plants succumbed, but the disease did not pass the sulphur barriers to attack the noninoculated plants on the other side. Neither the cotton roots nor the Phymatotrichum strands passed through the barriers. The soil within the barriers became as acid as pH 2.2, while that outside remained at the favorable pH of 7.5 to 8.

Single rows of graminaceous crops, such as sorghum and corn, tested in containers, were effective barriers. Cotton roots as well as Phymatotrichum strands failed to grow through the graminaceous root barriers, while roots of the latter grew out between the roots of the cotton plants but without contracting root rot.

Nursery plants as possible carriers of Phymatotrichum root rot. J. J. TAUBENHAUS and WALTER N. EZEKIEL.

Phymatotrichum root rot frequently appears on plantings in gardens, streets, or parks, where the disease was not known to occur before. In some such cases the disease originates from infected native vegetation or spreads from near-by infested areas. In other cases it has been suspected that root rot was brought in with the nursery plants. Balled and nonballed 2-year-old trees, from 2 nurseries where Phymatotrichum root rot had caused heavy damage, were set in cylinders and in the open, in a place where root rot had not occurred previously, although susceptible plants had been grown there constantly. Cotton was interplanted throughout. By midsummer, the cotton plants growing next to a balled hackberry tree in 1 cylinder succumbed to root rot. Likewise, a number of cotton plants growing next to 5 balled jujube plants planted in the open died from root rot only shortly before these jujube trees also succumbed. The presence of Phymatotrichum root rot in both cases resulted from the transmission of the disease on the roots or perhaps as sclerotia in the soil around these balled nursery plants.

An Actinomycete antagonistic to a Pythium root parasite of sugar cane. E. C. TIMS.

A number of Actinomycetes isolated from sugar-cane soils in southwestern Louisiana and grown on artificial media have proved antagonistic to a parasitic sugar-cane Pythium. One of these cultures proved especially active and was used in later tests. When applied to sterilized soil previously inoculated with Pythium under greenhouse conditions, the Actinomycete reduced the amount of root rotting in young cane and corn plants, and when grown in a nutrient solution produced a toxic principle that inhibited the growth of the sugar-cane Pythium. Filtrates from which the organism had been removed were toxic to the Pythium when added to string-bean-agar plates. This shows that the Actinomycete produces a toxic principle rather than exhausts the nutrients in the medium and starves the Pythium. Tests showed that change in the hydrogen-ion concentration of the nutrient solution was not the inhibiting factor. This toxic principle is apparently partially destroyed by heat of sufficient duration.

Behavior of mosaic in certain sugar-cane varieties in Louisiana. E. C. TIMS and C. W. EDGERTON.

Mosaic-infected P. O. J. 213 and P. O. J. 228 cane, observed at Baton Rouge since 1926, continued to produce some healthy stalks in 1931. However, the proportion of such healthy shoots produced from mosaic-infected seed was less in 1931 than in previous years. The percentage of infection in field plantings of P. O. J. 213 at Baton Rouge still remains small, 2 to 5 per cent.

A very different condition, however, occurs in cane from Reserve, a plantation about 50 miles south of Baton Rouge. The natural infection in P. O. J. 213 runs as high as 50 to 60 per cent, and there is considerable infection in Co. 281 and Co. 290, which are practically disease free at Baton Rouge. Mosaic-infected stalks of P. O. J. 213 and Co. 281 from Reserve, planted at Baton Rouge, produced only diseased shoots, all of which have remained infected throughout 1931. Also, 100 stalks of each of these 2 varieties showing mosaic in early summer were tagged and observed until planting time in October. They all showed the disease in typical form in late October.

This difference in behavior of mosaic in the 2 different sections suggests that there may be 2 strains of the sugar-cane-mosaic virus present in Louisiana.

Controlling bottom rot of lettuce by dusting. G. R. TOWNSEND.

Bottom rot (*Rhizoctonia solani*) usually destroys 30 per cent of New York's muck-grown lettuce. Losses to individuals of 85 per cent are common during warm, humid periods. No resistant strains are known. Breeding for escapement, crop rotation, careful sanitation, special cultivation, mulch papering, and green manuring have all failed as control measures.

Extensive experiments the past 3 years with fungicides have shown only mercury compounds to be efficient. Calomel, mercuric oxide, and mercuric chloride all injured the lettuce severely. The fungicide giving the best control of bottom rot with no injury to the crop was an ethyl mercury phosphate (DuBay No. 738). The best rate of application was about 25 pounds per acre. One application made 2 weeks before harvest gave practically complete protection. In 54 field experiments during 1930 and 1931 hand applications reduced the average incidence of bottom rot from 27 to 6 per cent.

Traction dusters have been built recently that blow the dust under the lower leaves. In 62 fields, covering 30 acres thus treated this year, the average incidence of bottom rot was reduced from 22.6 to 2 per cent. The average increase in yield was 160 crates per acre. Increases of over 500 crates have been secured.

Helminthosporium sigmoideum, the conidial stage of Sclerotium oryzae. E. C. TULLIS.

Sclerotium oryzae, which causes stem rot of rice, has been found to produce a conidial stage, identified as *Helminthosporium sigmoideum.* This conidial stage has been observed on agar cultures, on plants grown *in vitro,* and on plants grown in soil in the laboratory. Conidia of *H. sigmoideum* were found also in a herbarium specimen of *S. oryzae* collected in Italy by Briosi and Cavara. The genetic connection of the two stages has been demonstrated in inoculations and in cultural studies with both stages. Blue Rose rice seedlings grown aseptically on corn-meal agar in test tubes and inoculated with sclerotia of *S. oryzae* were killed by the fungus. Subsequently conidia of *H. sigmoideum* developed on these seedlings. These conidia germinated, infected rice seedlings, and produced in them and on agar the characteristic sclerotia of *S. oryzae.* The entire cycle, from sclerotia to mycelium to conidia to mycelium to sclerotia, has been followed under controlled conditions in the laboratory, and on plants grown aseptically on agar and in the soil. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas Agricultural Experiment Station.)

Ophiobolus oryzinus on rice in Arkansas. E. C. TULLIS.

Ophiobolus oryzinus, originally found in the Philippine Islands by C. F. Baker, on rotting rice straw, has been found to produce a disease of rice in Arkansas. In artificial inoculation experiments, *O. oryzinus* is pathogenic on Fortuna and Blue Rose rice plants in seedling and heading stages and on red rice plants in the seedling stage. Certain of the plants have been killed outright, while others have been injured through loss of leaf area. Red rice plants also have been found with their crowns invaded. Infected plants failed to tiller normally, producing tillers only after the heading of the first culm. Invasion of the host occurred by direct mycelial penetration of the epidermis of the basal leaves. Appressoria were formed and aided in the invasion of tissues. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas Agricultural Experiment Station.)

Two seed-transmitted ring-spot diseases of tobacco. W. D. VALLEAU.

Two ring spots of tobacco, designated green and yellow, are present in Kentucky. In one, chlorotic patterns are in shades of green; in the other, shades of green and yellow, with frequently large yellow or nearly white leaf areas. Both viruses are transmitted through tobacco seed in percentages up to 15. Affected seedlings of yellow ring spot turn yellow soon after germination. Of 24,060 seedlings grown from plants affected with yellow ring spot, 1,175, or 4.9 per cent, were positive a few days after germination. Seed transmission of green ring spot is difficult to detect except at low temperatures (50 to 65° F.), when the edges of the leaves of affected plants become chlorotic to necrotic. Tobacco seed probably has not played a part in the distribution of yellow ring spot, as affected seedlings grow so slowly that they would never be set. It may have played a part in the distribution of green ring spot. The flower parts of affected plants appear normal, but a majority of the pollen grains are somewhat small and sterile. In the absence of other symptoms, ring spot may be diagnosed by a study of pollen. Ovule sterility varies greatly when self-pollination occurs, but this may be due to abortive pollen.

The development of Cercospora leaf spot on sugar beets grown under different cultural conditions. EDGAR F. VESTAL.

The percentage of occurrence of Cercospora leaf spot in rows spaced 22 inches apart was on August 30, with 12-inch blocking, disease-free 26.6, light infection 28.5, heavy infection 30.7, dead 14.4; with 20-inch blocking, disease-free 30.6, light infection 56.9, heavy infection 3.9, dead 8.4. On September 30 the percentage occurrence in 12-inch blocking was disease-free 28.2, light infection 4.8, heavy infection 16.8, dead 50.2; with 20-inch blocking, disease-free 29.6, light infection 6.7, heavy infection 29.6, dead 34.0.

The early reading shows nearly 8 times as many heavily infected and ¾ more dead leaves among the 12-inch than among the 20-inch blocking. The later reading showed nearly ½ more dead leaves and half as many heavily infected leaves in the 12-inch blocking as compared with the 20-inch. The epidemic was delayed about 1 month by the wider spacing, affording better conditions for development of these beets. The yields were 13.5 and 15.4 tons per acre, respectively, for the 12- and 20-inch blocking. The sugar content and purity were nearly equal for both.

A similar comparison between the 24- and 16-inch spacings showed nearly the same results as above.

Decomposition of the safranin precipitate of mosaic virus of tobacco. CARL G. VINSON.

The safranin precipitate of the virus of tobacco mosaic has been satisfactorily decomposed by adding Lloyd's alkaloidal reagent to it. The mixture is agitated at intervals for about ½ hour, and then is centrifuged at high speed. One treatment with the reagent may suffice to remove all the safranin from the supernant liquid, but, if not, the treatment is repeated until all the dye is removed. After removal of the safranin the supernatant liquid is usually more highly infectious than the original juice from which the safranin precipitate is prepared. This method of purification is simple and rapid and involves only very mild treatment of the virus.

Comparison of juices from diseased and healthy tobacco plants. CARL G. VINSON and EDGAR J. GILDEHAUS.

The virus of tobacco mosaic can be precipitated from juice of diseased plants by addition of a solution of lead acetate. After washing the precipitate with a $M/_3$ solution of primary potassium ortho phosphate the virus can be freed from the precipitate by means of a dilute solution of potassium hydrogen phosphate, which is nearly neutral in reaction. Such a solution of the virus is very infectious and contains Kjeldahl nitrogen. The same procedure applied to juice from healthy plants yields a solution that contains no Kjeldahl nitrogen.

Gibberella moniliformis on corn plants in the field. R. K. VOORHEES and A. H. EDDINS.

Gibberella moniliformis, the perfect stage of *Fusarium moniliforme,* has been found occurring naturally on corn leaf sheaths in Florida. Macroscopically, the perithecia of this fungus are similar to those of *G. saubinetii* but are characteristically less cespitose on this host. Wineland reported the development of perithecia of *G. moniliformis* in pure culture. In the present investigations perithecia of the fungus developed in cultures from conidia of *F. moniliforme.* Perithecia also developed on cornstalks inoculated with single and multisporous conidial cultures. Cultures from single ascospores have produced both microconidia and macroconidia of *F. moniliforme.*

Particle size of the virus of tobacco mosaic in purified solutions. JOHN G. WAUGH and CARL G. VINSON.

Results of preliminary experiments indicate that the radius of the virus particle in our purified solutions is less than 5 millimicrons.

The toxicity of naphthalene for fungi of the Sclerotium rolfsii type. FREEMAN WEISS and E. L. EVINGER.

Naphthalene suspended in corn-meal agar in minute crystals is toxic to cultures of *Sclerotium rolfsii* grown on this medium. The germination of sclerotia and growth of mycelium are retarded by concentrations as low as 1 part of naphthalene to 10,000 (weight basis) of medium. A concentration of 1: 5,000 entirely prevents growth. Sclerotia kept on agar plates of greater than 1: 5,000 naphthalene concentration do not revive when transferred to naphthalene-free agar. Direct contact with naphthalene crystals is not requisite for toxic action, as naphthalene vapor kills sclerotia in 3 to 4 days' exposure at 30° C.

In preliminary tests with potted plants, and in field plots, repression of *Sclerotium rolfsii* by naphthalene, without toxic effects on the crop plants tested, is indicated. As a soil fungicide naphthalene possesses the additional features of exceptionally low cost (2 to 4 cents a pound) and effective distribution in soil because of its volatility. Preliminary trials with other soil-inhabiting fungi, such as Rhizoctonia, Fusarium, and Penicilium, indicate that naphthalene is somewhat fungicidal generally. Naphthalene already has an accepted standing as an acaricide and rodent repellent.

Control of celery mosaic by eradicating wild hosts. F. L. WELLMAN.

The celery mosaic that occurs in Florida is not seed-borne but persists from season to season in *Commelina nudiflora* and other wild hosts. Insects, chiefly *Aphis gossypii*, migrating from infected weeds to celery, can carry the mosaic virus at least 600 feet, though initial infection occurs more commonly within 50 feet of wild hosts. Four closely adjacent celery fields were used in the eradication experiments during the winter of 1930–31. In fields A and B weeds were removed from ditch banks and around buildings and bushes shortly after celery was transplanted. In field C eradication was practiced 2 months after it was begun on A and B; in field D no weeds were removed. In parts of field A there was more than 60 per cent of infection in 1929–30. In 1930–31 the disease occurrence in these same areas was reduced to around 6 per cent. In field B practically the same results were obtained. In fields C and D nearly as heavy infection occurred in 1930–31 as in 1929–30, though the somewhat cooler season of 1930–31 seemed to inhibit its appearance to a considerable extent.

Wilt resistance of Pride of Muscatine (K–S4) watermelons on heavily versus moderately infested soil. J. J. WILSON.

A comparison was secured in 1931 of the response of the wilt-resistant variety, Pride of Muscatine (K–S4), in 2 fields infested with *Fusarium niveum*. Field No. 1 had been cropped to melons 6 consecutive years, while No. 2 had not. On July 3, Pride of Muscatine showed 25 per cent wilt in field No. 1 and 3.5 per cent in field No. 2. Stand counts of plants were continued until October 5, at which time 43 per cent had died in field No. 1 and 11 per cent in field No. 2. The variety Kleckley Sweet, used as checks, showed 68 per cent wilt on July 3, when the plants had to be sacrificed to prevent cross pollination. The response of Pride of Muscatine in field No. 2 was typical of the performance of this variety in 1931 when grown on 750 acres of wilt-infested soil in Muscatine County.

During the first 2 weeks following emergence it was noted that 17 per cent of the Pride of Muscatine plants and 31 per cent of the Kleckley Sweet plants had died in field No. 1. This heavy mortality of seedlings has occurred during the past 5 years in 3 experimental fields, continuously cropped to melons, where the soil is very heavily infested with *Fusarium niveum*.

Ampelomyces quisqualis on clover mildew. CECIL E. YARWOOD.

During the late summer and fall of 1931, *Ampelomyces quisqualis,* a fungous parasite of powdery mildews, was apparently responsible for the almost complete killing out of clover mildew in experimental plots of red clover. It also was abundant on other mildews and has been isolated in pure culture from the following hosts: *Erysiphe polygoni* on red clover, *Microsphaera alni vacinii* on catalpa, *Sphaerotheca pannosa* on rose, *Erysiphe cichoracearum* on zinnia, *E. cichoracearum* on *Ambrosia artemisiifolia, Microsphaera alni* on *Lonicera* sp., and an undetermined mildew species on *Plantago major.* The isolations from the first five hosts show distinguishing cultural characteristics on agar. Inoculations were performed by atomizing a suspension of Ampelomyces spores onto vigorously growing mildew colonies. Infection of clover mildew has resulted from inoculation with cultures from clover, catalpa, and rose mildews. The optimum temperature for spore germination of Ampelomyces and for infection of clover mildew is about 25° C. Pycnidia are produced in abundance in about 6 days following inoculation of living mildew, but growth and pycnidium formation are very slow in agar cultures. At 25° C. growth and conidium formation of clover mildew colonies may be completely stopped in about 8 days after inoculation with Ampelomyces. (Cooperative investigations, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Purdue University Agricultural Experiment Station.)

Reversible phototropism of the germ tubes of clover powdery mildew. CECIL E. YARWOOD.

In high light intensities, such as full exposure to day-light, even of a cloudy day, the tubes of powdery mildew of red clover are positively phototropic. In relatively low light intensities they are negatively phototropic. Appressoria are produced by the germ tubes due to contact stimuli, their formation is dependent to certain extent on the vigor of the phototropism, and they may be formed by positively- or negatively-phototropic germ tubes. The percentage of germination is much higher in light than in darkness, but good infection is secured on the host, either in light or in darkness. (Cooperative investigations, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Purdue University Agricultural Experiment Station.)

Lubricating-oil sprays and their effect on potatoes. PAUL A. YOUNG.

Oil sprays control potato aphids cheaply. In a field 103 hills of Triumph potatoes, sprayed with a 2 per cent cresoap emulsion of a 90 per cent non sulphonatable oil, yielded 9.1 per cent less than the checks; 55 other hills sprayed with a 1 per cent emulsion of this oil yielded 5 per cent more than the checks. These differences were insignificant. Similar vines sprayed with 8 to 16 per cent oil emulsions produced few tubers and the vines died prematurely. Pure oils and 4 to 16 per cent emulsions caused the following symptoms in potato leaves: translucent spots; black to brown spots; epiphyllous white spots normal or brown hypophyllously; epiphyllous blackening and browning of veins; and rolling, wilting, and yellowing. Oils injured young leaves more than old ones. Oil partially girdled the bases of potato stems, causing some aerial tubers. N-decane quickly caused translucent and brown leaf spots. One to 8 per cent cresoap emulsions freeze, ice crystals forming between layers of oil and air. Blocks and lamellae of ice form between oil layers and networks. Strong cresoap emulsions reform in melting. Cresoap miscible oil, touching water, instantly formed emulsified oil droplets 0.2 to 3 μ in diameter.

SOME NUTRITIONAL DISORDERS IN CORN GROWN IN SAND CULTURES[1]

N. A. Pettinger, R. G. Henderson, and S. A. Wingard[2]

INTRODUCTION

During the course of some sand-culture experiments with corn in the summer of 1930, there occurred several different types of disorders in the plants that have since been traced to nutritional causes. Chief among these were three distinct types of chlorosis, two of which appear to be different from the several types previously reported in the literature for corn. It was noticed also that the plants were developing sterile tassels and, therefore, would not be able to reproduce normally. These abnormal developments in the leaves and tassels led to an investigation of their causes during the fall of 1930 and summer of 1931, and the present paper reports the results obtained in these studies.

Disorders in plants usually are classified under three types, namely: (1) hereditary, (2) infectious, and (3) nutritional. The corn used in the studies here reported was an F_1 cross of two inbred strains of the Reid Yellow Dent variety grown at Bloomington, Ill. The F_1, as well as the parental strains, has never developed chlorosis or been sterile when grown in soil, which indicates that the maladies observed in the sand cultures were not of the hereditary type. On some of the leaves, the chlorotic areas appeared to be harboring pathogenic organisms, which suggested that the trouble might be of an infectious nature. There was also the possibility that the nutrient solutions applied to the sand were either deficient or toxic in one or more of the nutrients required by the plant or that the nutrients were not applied in the correct proportions with respect to each other. In attempting to learn the cause or causes of these disorders, therefore, it was necessary to attack the problem from both the infectious and nutritional standpoints.

DESCRIPTION OF DISORDERS

Types of chlorosis: The leaves of the plants grown in the sand cultures were not uniform with respect to their chlorophyll deficiencies. Casual observation of the chlorotic areas showed the presence of two distinctly different types of chlorosis, but closer observation and study of one of these

[1] Joint paper from the Departments of Agronomy and Plant Pathology of the Virginia Agricultural Experiment Station and paper No. 79 from the Department of Botany and Plant Pathology.

[2] Associate Agronomist, Assistant Plant Pathologist, and Plant Pathologist, respectively.

types disclosed minor differences in the outlines of the chlorotic areas, which indicated that there were perhaps three types present. This suspicion was confirmed by the fact that the differences observed in the leaves have now been traced to different causes. To facilitate reference to these different types of chlorosis, they will henceforth be designated in this paper as types A, B, and C.

Type A appears to be identical to that described by Jones (5), which was found to be caused by a deficiency of magnesia. The affected areas

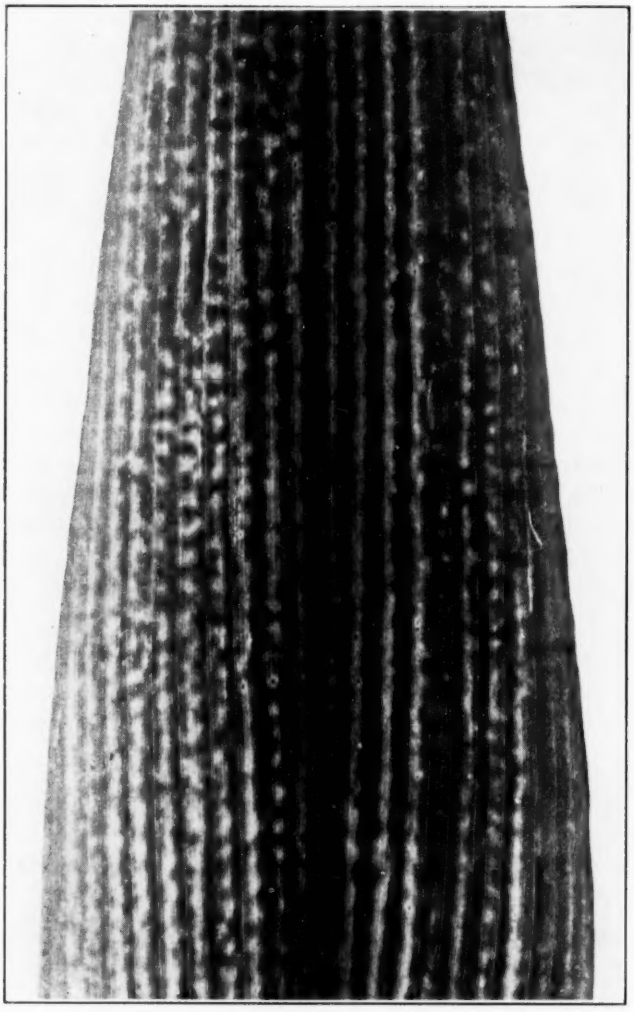

FIG. 1. Leaf from corn plant grown in sand culture that was deficient in magnesuim, showing type-A chlorosis.

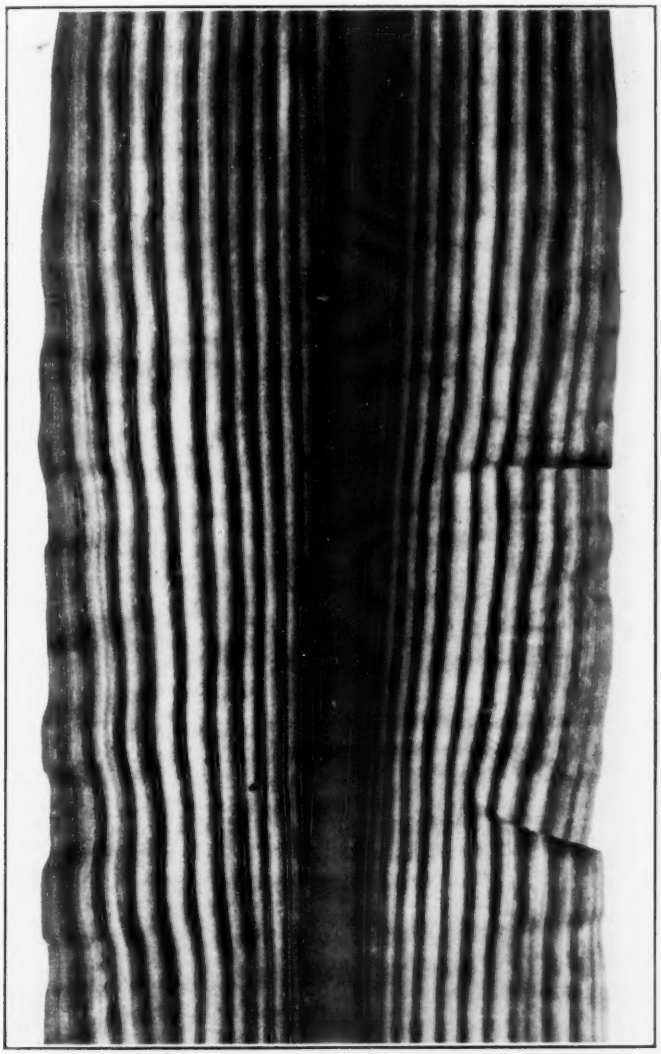

FIG. 2. Leaf from corn plant grown in sand culture that received an excess of sodium, showing type-B chlorosis.

appeared as long, narrow, chlorotic streaks that are more or less continuous from the base to the tip of the leaves. The streaks, however, are very irregular along the margins and in some cases are broken up into chains of alternate green and chlorotic splotches (Fig. 1). In contrast to this, the streaks in type B are continuous, with very regular margins, and extend from vein to vein the full length of the leaf (Fig. 2). In the early stages

of growth of the plants, these types of chlorosis were characterized by alternate streaks of light green and dark green in the leaves; the light green being in the intervascular tissues and the dark green in the vascular regions. As growth progressed, the light green of the intervascular tissues was gradually lost, making the chlorotic streaks more apparent. In the advanced stages of development of type A necrosis set in, causing the central parts of the chlorotic streaks to become brown.

Type C was quite distinct in appearance from either A or B and could readily be distinguished from them by a casual observer. This type had the appearance of a bacterial leaf spot and was thought at first to be of bacterial origin. It is also similar to a hereditary type of chlorosis described by Koehler and Holbert (6) and designated as "dying between the veins." The white or chlorotic spots that characterized this trouble first appeared on the leaves when the plants were about 3 weeks old. At this time the affected areas were small, but they enlarged as the leaves grew. At all times, however, they were confined to the tissues between the veins. The

FIG. 3. Leaves from corn plants grown in sand cultures that were deficient in minor elements, showing type-C chlorosis, which is attributed to a deficiency of manganese.

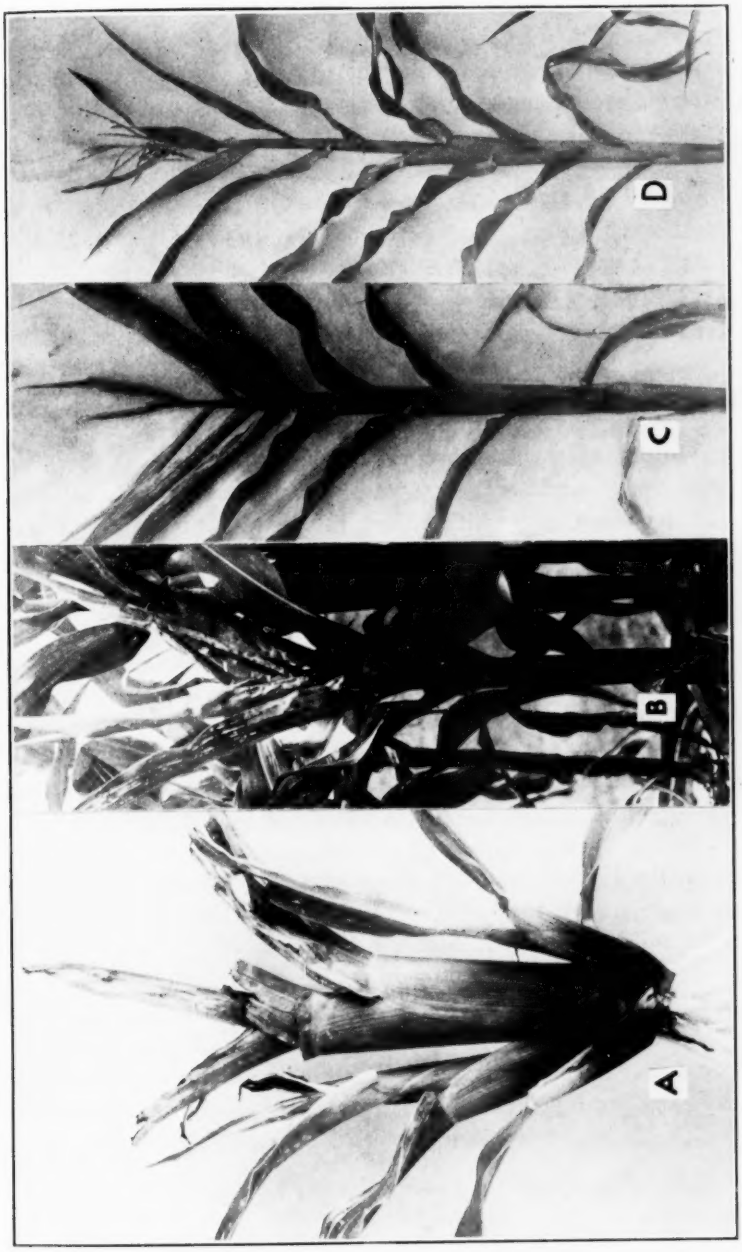

FIG. 4. Abnormal developments in corn plants grown in sand cultures that received no minor elements. A. Affected in seedling stage. B and C. Affected just prior to tasseling. Note normal growth made during earlier stages. D. Plant that developed a trace of chlorosis at tasseling time.

green pigment disappeared very rapidly and completely, so that all of the affected areas appeared translucent from the beginning. These chlorotic areas were irregular in outline and more or less longitudinal in shape. When abundant in the young leaves they elongated to such an extent as to coalesce, thereby forming long, continuous chlorotic streaks in the more advanced stages (Fig. 3 and Fig. 6, A). When these areas appeared at some distance from each other, they often failed to coalesce on enlargement, giving the leaf a "buck-shot" appearance. As the season progressed the chlorotic tissues began to break down and ceased to function in the activities of the plant (Fig. 3). This resulted in streaks of dead, brown tissue in the center of the chlorotic areas. The necrotic tissue sometimes fell away, leaving holes in the leaves. In the more severe cases the chlorotic areas were covered by a clear gelatinous substance.

Dwarfing: All types of chlorosis were accompanied by reduced growth. The plants affected with types A and B were only slightly shorter, the internodes and leaves retaining their normal proportions throughout the plant. In type C, however, varying degrees of distortion of growth occurred, depending on the stage of growth in which the plant became affected. When affected in the early seedling stage the growing points were either injured or killed, resulting in stunted and deformed stalks and leaves, the leaves being only ragged vestiges a few inches in length (Fig. 4, A). Such plants also showed a tendency to tiller. In plants in which the cause of the disorders did not become acute until the later stages of growth, the lower parts of the plant developed normally but the upper leaves and internodes were malformed. The upper internodes were very much shortened, giving the leaves a bunched appearance (Fig. 4, B, and C). The leaves also showed a tendency to curl inwardly at the margins and were usually very chlorotic.

Sterility: In the 1930 cultures all the plants that reached the shooting and tasseling stage before the experiment was discontinued proved to be sterile. In the milder cases of chlorosis tassels were produced but the flowers were sterile (Fig. 4, D), while in severe cases no tassels or shoots whatever developed (Fig. 4, B, C). The latter condition was associated more often with the type-C chlorosis than with either of the other types. Plants that were only slightly chlorotic with stalks and leaves of apparently normal proportions developed tassels and shoots that might have been functional had the plants been allowed to remain longer in culture.

CONDITIONS UNDER WHICH THE CORN WAS GROWN

The plants were grown in galvanized iron cans, 18 in. in diameter and 24 in. deep, filled with quartz sand to within an inch of the top. The cans

were coated with paraffin on the inside to prevent zinc toxicity and were provided with drainage at the bottom. Insulation against air temperatures was attained by inserting the cans in a long wooden box 18 in. above the ground and filling in around them with soil. Eight kernels were planted in each can and the seedlings thinned down to 4 when a week old. The 1930 cultures were planted on June 6 and terminated during the second week of August, while the 1931 cultures were planted on May 18 and terminated during the first week of August. At the time the experiment was terminated in both years the plants were beginning to form shoots and tassels. The experiment was conducted in an open field.

The purpose of the sand-culture tests herein described was to determine the effect of different concentrations of the nutrients supplied to the sand on the composition of certain plant parts. The plan was to vary one element at a time, keeping all other elements constant in the cans in which the element in question varied in concentration. Thus, in the cans where calcium was applied in different concentrations, magnesium, nitrogen, phosphorus, potassium, iron, and sulphur were applied at their respective concentrations to all cans throughout the experiment. Five elements were varied in this manner, namely, magnesium, calcium, nitrogen, phosphorus, and potassium. Each of these elements, in turn, was applied in 5 different concentrations, making a total of 25 different nutrient cultures. The first concentration was made "very low," the second "low," also, but higher than the first, the third "medium," the fourth "high," and the fifth "very high." The medium concentration, which was used for a standard, was based (with slight modifications) on the nutrient solutions used by Duley and Miller (2) in similar work at the Missouri station. Their solution was adopted as a standard because of the success they had in securing good growth. Each solution was tested in duplicate.

The concentration of each of the stock nutrient solutions used is as follows:

$MgSO_4 \cdot 7H_2O$	100	gms. in 2 liters of distilled water.
$Ca(NO_3)_2 \cdot 4H_2O$	250	" " " " " " "
$NaNO_3$	136.7	" " " " " " "
KCl	25	" " " " " " "
$CaCl_2 \cdot 2H_2O$	140	" " " " " " "
KH_2PO_4	50	" " " " " " "
$NaH_2PO_4 \cdot H_2O$	50	" " " " " " "
$FeCl_3 \cdot 6H_2O$	8	" " " " " " "

The quantities of each stock solution applied to the different cultures in 1930 are given in table 1. The same quantities of these solutions were added in 1931, and, in addition, boron, zinc, copper, and arsenic were added at the rate of 0.5 part per million and manganese at the rate of 1.0 p.p.m. The

TABLE 1.—*Cubic centimeters of stock nutrient solutions added to 4 liters of water*

	Cans 1 and 2	Cans 3 and 4	Cans 5 and 6	Cans 7 and 8	Cans 9 and 10
			Magnesium Series		
$MgSO_4 \cdot 7H_2O$	1	5	10	15	20
$Ca(NO_3)_2 \cdot 4H_2O$	20	20	20	20	20
KCl	20	20	20	20	20
KH_2PO_4	20	20	20	20	20
$FeCl_3 \cdot 6H_2O$	2	2	2	2	2
			Calcium Series		
$MgSO_4 \cdot 7H_2O$	10	10	10	10	10
$Ca(NO_3)_2 \cdot 4H_2O$	4	10	20	20	20
$NaNO_3$	32	20	0	0	0
KCl	20	20	20	20	20
$CaCl_2 \cdot 2H_2O$	0	0	0	20	60
KH_2PO_4	20	20	20	20	20
$FeCl_3 \cdot 6H_2O$	2	2	2	2	2
			Nitrogen Series		
$MgSO_4 \cdot 7H_2O$	10	10	10	10	10
$Ca(NO_3)_2 \cdot 4H_2O$	4	10	20	20	20
$NaNO_3$	0	0	0	40	120
KCl	20	20	20	20	20
$CaCl_2 \cdot 2H_2O$	16	10	0	0	0
KH_2PO_4	20	20	20	20	20
$FeCl_3 \cdot 6H_2O$	2	2	2	2	2
			Phosphorus Series		
$MgSO_4 \cdot 7H_2O$	10	10	10	10	10
$Ca(NO_3)_2 \cdot 4H_2O$	20	20	20	20	20
KCl	40	31	20	20	20
KH_2PO_4	2	10	20	20	20
$NaH_2PO_4 \cdot H_2O$	0	0	0	10	20
$FeCl_3 \cdot 6H_2O$	2	2	2	2	2
			Potassium Series		
$MgSO_4 \cdot 7H_2O$	10	10	10	10	10
$Ca(NO_3)_2 \cdot 4H_2O$	20	20	20	20	20
KCl	4	10	20	80	160
KH_2PO_4	4	10	20	20	20
$NaH_2PO_4 \cdot H_2O$	16	10	0	0	0
$FeCl_3 \cdot 6H_2O$	2	2	2	2	2

compounds in which these elements were added are listed in table 3. In 1931 an attempt was also made to raise the nitrogen level of the experiment by adding 10 cc. of sodium nitrate to all cultures except those made deficient in nitrogen. After 3 applications, however, this practice was discontinued because of the appearance of type B chlorosis already described.

When it was desired to add nutrients to the sand the specified amounts of the various stock solutions were first mixed together in a beaker, then added to 4 liters of distilled water, and sprinkled over the surface of the sand. The chemicals used were of the usual "C. P." grade.

HYPOTHESES TO ACCOUNT FOR THE DISORDERS

When the type-C chlorosis was first observed, it was thought to be a bacterial trouble. This was indicated by the presence of a gelatinous substance on the surface of the chlorotic areas, which resembled the exudate common to many bacterial infections on plants. Microscopic examination showed that this material contained numerous bacteria. Isolations yielded two types of bacterial colonies, one of which was bright yellow, the other cream-color. Both organisms, however, proved to be noninfectious on healthy corn grown in soil in the greenhouse. Repeated inoculations by needle punctures and swabbing of the leaf surfaces gave negative results. Similar inoculations made directly from infusions of the affected tissues in nutrient broth also gave negative results, showing conclusively that the bacteria present in the chlorotic areas were only secondary. A careful examination of the roots of the affected plants showed them to be free from bacterial and fungous infection. In contrast to the type-C chlorosis, types A and B did not appear to be of an infectious nature. Furthermore, the sterility and stunting showed no evidence of having been caused by pathogenic organisms.

After eliminating the possibility that these disorders might be of either hereditary or pathogenic origin, it became necessary to consider them from the nutritional standpoint. This naturally led to a close examination of all the cultures for possible relations between the presence and severity of the chloroses and other disorders and the nutrients applied to the sand.

Chloroses: It was found that the type-A chlorosis (Fig. 1) occurred only in the cultures that received insufficient magnesium. Cans 1 and 2 (table 1) of the magnesium series received very small amounts of magnesium and supplied the excellent specimen shown in figure 1. The plants grown in cans 3 and 4 of this series were nearly normal in color; the intervascular tissues of the leaves were light-green but were not so devoid of chlorophyll as those of cans 1 and 2. Since this type of chlorosis occurred only in the magnesium-deficient cultures, it is apparent that the deficiency of magnesium was the cause of this type of chlorosis.

The type-B chlorosis was found to occur in all cultures where sodium was added in the nutrient solutions. In the calcium-deficient cultures of the calcium series sodium was added as sodium nitrate to replace the nitrate lost by withholding the calcium of calcium nitrate. In the nitrogen series this type of chlorosis appeared in the cultures that received an excess of nitrates, the nitrate over and above the basal amount of calcium nitrate being added as sodium nitrate. In the excess-phosphate cultures sodium was added as sodium di-hydrogen phosphate to increase the phosphate above that supplied by the basal quantity of potassium di-hydrogen phosphate. Sodium was also added as sodium di-hydrogen phosphate in the potassium series to replace the phosphate lost when potassium di-hydrogen phosphate was withheld to make potassium subnormal in the potassium-deficient cultures. All of these cultures showed the smooth-margin, continuous streaking shown in figure 2 and indicate that this type of chlorosis was caused by an excess of sodium in these cultures. This belief is strengthened by the observation that when an attempt was made to raise the nitrogen level of the experiment by adding 10 cc. of the sodium-nitrate stock solution to all cultures except those deficient in nitrogen, this same type of chlorosis made its appearance in a mild form in nearly all of the cultures. Hence, after three successive additions of nutrients with the sodium nitrate included, this practice was discontinued. The plants gradually regained a normal green color in the intervascular tissues of the leaves, which confirms the belief expressed above that this type of disorder resulted from sodium toxicity.

The type-C chlorosis (Fig. 3) occurred throughout all of the 1930 cultures except those that received an excess of calcium or a deficiency of nitrogen. Occasionally single plants in other cultures were free from this type of chlorosis, even though other plants in the same culture were affected.

The fact that the excess-calcium and nitrogen-deficient cultures were free from the type-C chlorosis suggests the possibility that a high-calcium content or a low-nitrogen content in the nutrient solutions might explain the lack of chlorosis in these cultures. However, a close study of the solutions applied to the other cultures of the experiment shows that neither of these situations can explain the apparently normal cultures. On the other hand, there is a close correlation between the ratio of calcium to nitrogen and the presence or absence of chlorosis in the leaves; the normal cultures having ratios above 1.5 : 1 and the chlorotic ones ratios of 1.5 : 1 or lower. This indicates that the ratio between these elements may have been of more importance than the actual amounts added. In what way a wide Ca : N ratio might operate to prevent chlorosis is not apparent at present; the close correlation, however, may be significant.

Brown (1) has reported chlorosis in wheat grown in sand cultures in which calcium nitrate and potassium di-hydrogen phosphate were applied

TABLE 2.—*Effect of nutrient ratios on the development of chlorophyll in the leaves of corn plants grown in sand cultures*

Nutrient concentration					Ratio of			Chlorosis
Magnesium	Calcium	Nitrogen	Phosphorus	Potassium	Ca : Mg	Ca : N	$Ca(NO_3)_2$: KH_2PO_4	
Very low	Medium	Medium	Medium	Medium	60.0 : 1	1.5 : 1	2.1 : 1	Present
Low	"	"	"	"	12.0 : 1	1.5 : 1	2.1 : 1	"
Medium	"	"	"	"	6.0 : 1	1.5 : 1	2.1 : 1	"
High	"	"	"	"	4.0 : 1	1.5 : 1	2.1 : 1	"
Very high	"	"	"	"	3.0 : 1	1.5 : 1	2.1 : 1	"
Medium	Very low	"	"	"	1.2 : 1	.3 : 1	.4 : 1	"
"	Low	"	"	"	3.0 : 1	.7 : 1	1.0 : 1	"
"	Medium	"	"	"	6.0 : 1	1.5 : 1	2.1 : 1	"
"	High	"	"	"	11.0 : 1	2.6 : 1	2.1 : 1	Trace
"	Very high	"	"	"	21.0 : 1	5.0 : 1	2.1 : 1	Absent
"	Medium	Very low	"	"	6.0 : 1	6.2 : 1	20.6 : 1	"
"	"	Low	"	"	6.0 : 1	2.6 : 1	4.1 : 1	"
"	"	Medium	"	"	6.0 : 1	1.5 : 1	2.1 : 1	Present
"	"	High	"	"	6.0 : 1	.7 : 1	2.1 : 1	"
"	"	Very high	"	"	6.0 : 1	.3 : 1	2.1 : 1	"
"	"	Medium	Very low	"	6.0 : 1	1.5 : 1	2.1 : 1	"
"	"	"	Low	"	6.0 : 1	1.5 : 1	2.1 : 1	"
"	"	"	Medium	"	6.0 : 1	1.5 : 1	2.1 : 1	"
"	"	"	High	"	6.0 : 1	1.5 : 1	2.1 : 1	"
"	"	"	Very high	"	6.0 : 1	1.5 : 1	2.1 : 1	"
"	"	"	Medium	Very low	6.0 : 1	1.5 : 1	2.1 : 1	"
"	"	"	"	Low	6.0 : 1	1.5 : 1	2.1 : 1	"
"	"	"	"	Medium	6.0 : 1	1.5 : 1	2.1 : 1	"
"	"	"	"	High	6.0 : 1	1.5 : 1	2.1 : 1	"
"	"	"	"	Very high	6.0 : 1	1.5 : 1	2.1 : 1	"

in approximately equal molecular proportions. He states that this was caused by nutrient balance and increased permeability. That the disorders observed by the writers were not due to this cause is shown by the $KH_2PO_4 : Ca(NO_3)_2$ ratios given in table 2. Nor do the Ca : Mg ratios (Table 2) explain the condition of any of the cultures. The presence of the type-A chlorosis in the magnesium-deficient cultures is believed to be the result of insufficient magnesium rather than an unfavorable Ca : Mg ratio. The evidence indicates that in the case of calcium and magnesium the amounts added were of more importance than the ratio between these elements.

Table 1 shows that the chlorosis-free cultures of 1930 were the only ones that received calcium chloride in the nutrient solutions. This material was added to cultures in the calcium series to build up an excess of calcium and in the nitrogen series to make up for the loss of calcium occasioned by withholding calcium nitrate from the nitrogen-deficient cultures. That the calcium thus added does not account for the lack of chlorosis has been pointed out above. It is also unlikely that the additional chlorine added in the calcium chloride prevented the development of chlorosis, for chlorine also was made high in the potassium series by adding additional potassium chloride to the excess-potassium cultures. These cultures, in spite of the high chlorine content of their nutrient solutions, were as chlorotic as most of the others.

Since the evidence just presented concerning some of the major plant-food elements is more or less ineffective in explaining the occurrence of the type-C chlorosis in the 1930 cultures, the writers turned their attention to a consideration of the so-called minor elements, manganese, zinc, copper, arsenic, boron, etc. These elements were omitted because it was believed that sufficient amounts of each would be included as impurities in the other chemicals and sand to supply the needs of the plants. However, when the chlorosis made its appearance, it was decided to start additional cultures to determine whether the lack of minor elements might be responsible for the chlorosis. These tests were begun in the greenhouse in September, 1930. Corn from the same lot of seed as was used in the field cultures was planted in the same kind of sand. The cultures remained in the greenhouse until after the seed had germinated. They were then placed outside, so that the plants would secure better light for growth. At the age of 3 weeks, the seedlings began to show chlorophyll breakdown. Sixteen of them were then transplanted individually to 3-gal. earthenware pots filled with sand. One-half of these pots were then supplied with the basal nutrient solution only, while the others were given the minor elements listed in table 3, in addition to the basal nutrients. Several seedlings also were transplanted to pots filled with a fertile loam soil.

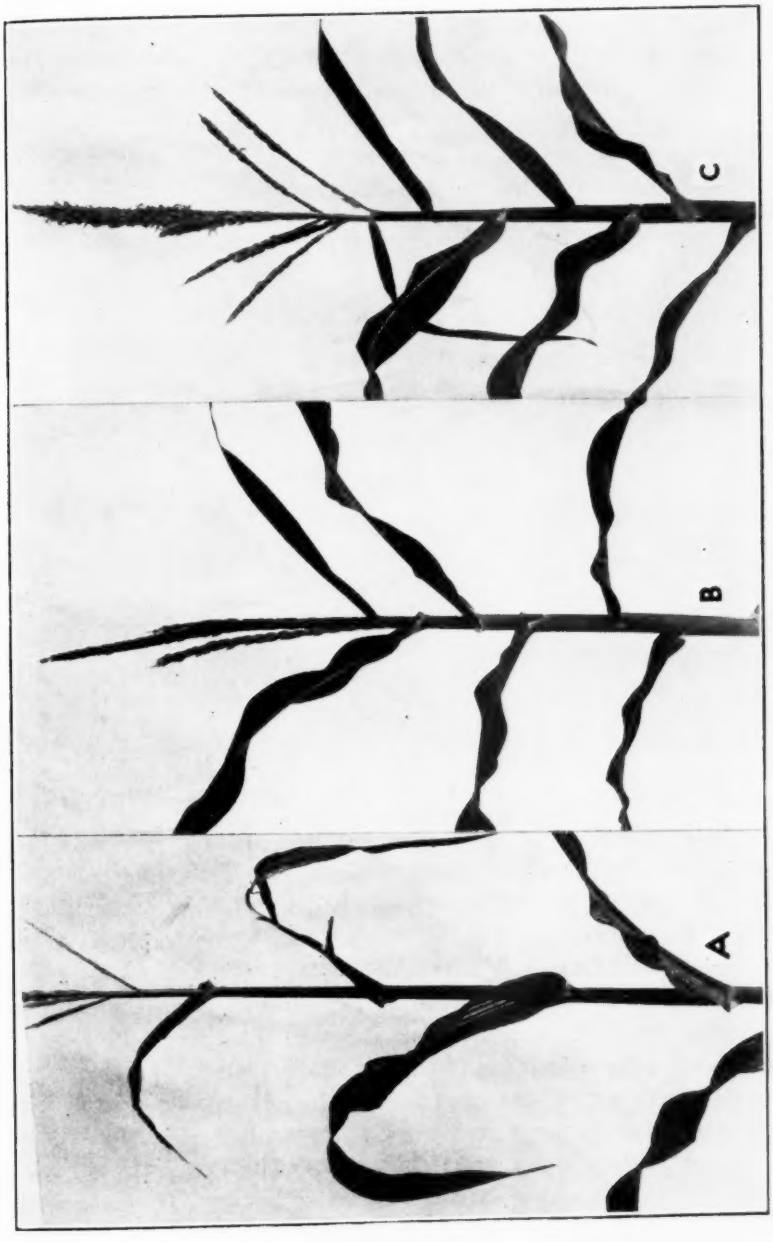

FIG. 5. A. A plant grown in sand that received no minor elements, showing type-C chlorosis on leaf blades and a sterile tassel. B. Grown in sand without minor elements until chlorosis began to appear, then transplanted to sand supplied with minor elements. C. Grown in sand without minor elements until chlorosis began to appear, then transplanted to a fertile loam soil. Note the freedom from chlorosis and the presence of fertile tassels in B and C.

The results of this test indicate that the chlorosis observed in the field cultures and reproduced in the greenhouse cultures was caused by a lack of one or more of the minor elements. Representative plants of the sand and soil cultures are shown in figure 5, and leaves of the same plants are shown in figure 6. The cultures that received basal nutrients only were

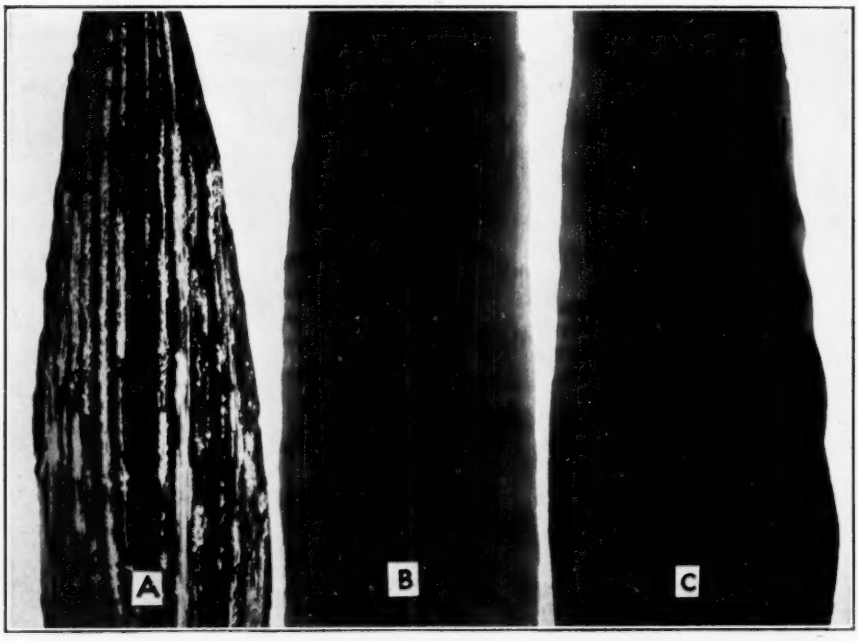

FIG. 6. A, B and C. Leaves from plants shown in figure 5 A, B and C, respectively.

uniformly chlorotic (Fig. 5, A; Fig. 6, A), while those that received the minor elements, in addition to the basal nutrients, were free from chlorosis (Fig. 5, B; Fig. 6, B). The plants grown in soil were also free from any chlorophyll defects (Fig. 5, C; Fig. 6, C) and were a darker green than those grown in sand either with or without the minor elements.

Owing to the pressure of other work, it was impossible to extend these tests to determine which of the five minor elements added were effective in preventing the chlorosis. Judging from information available concerning the functions of zinc, copper, arsenic, and boron in plants, it is improbable that any of these elements prevented the development of chlorosis. Concerning manganese, however, there is considerable evidence in the literature to indicate that the chlorosis observed in the sand cultures described here may have been due to a lack of sufficient available manganese. Thus, McHargue (8, 9) found that a lack of sufficient manganese caused chlorosis in

a number of economic plants and concluded that manganese plays a rôle of importance equal to that of iron in the synthesis of chlorophyll and carbon assimilation. Lee and McHargue (7) have recently described a type of chlorosis occurring in sugar cane grown in Hawaii found to be due to manganese deficiency. They successfully corrected the disease by applying manganous sulphate to the leaves. Gilbert, McLean, and Hardin (3) have been successful in correcting chlorosis in spinach by applying manganese solutions to the leaves. Skinner and Ruprecht (10) report that crops growing in certain soils of the Florida Everglades are generally chlorotic unless manganese is applied in a soluble form. These investigations, as well as others that might be cited, show quite conclusively that manganese is essential to chlorophyll formation.

That the occurrence of the type-C chlorosis in the 1930 cultures resulted from a deficiency of manganese and that the correction of this type of chlorosis in the sand cultures in the greenhouse was due to the addition of manganese rather than boron, zinc, copper, or arsenic were definitely shown by the 1931 sand cultures in the field. Since the greenhouse tests had indicated that the addition of the minor elements were effective in preventing the type-C chlorosis, the 1931 field cultures were all supplied with the

TABLE 3.—*Composition of stock solutions of minor elements and amounts added to nutrient solutions applied to sand cultures*

Chemicals used	Amount dissolved in 2 liters of water (grams)	Amount of stock solution added to 4 liters of water (cc.)
As_2O_3	0.528	10
$ZnSO_4 \cdot 7H_2O$	1.76	10
$MnSO_4 \cdot 4H_2O$	1.62	10
H_3BO_3	2.25	10
$CuSO_4 \cdot 5H_2O$	1.572	10

amounts indicated in table 3 at each application of nutrients. When the plants were about a month old, however, many of them began to show the development of typical spots of the type-C chlorosis. Acting on the theory that manganese was deficient rather than any of the other minor elements added, the manganese ration was increased tenfold, from 0.5 to 5.0 parts per million. After two applications at this rate the ration was reduced to 1.0 part per million for the remainder of the experiment. This treatment not only restored chlorophyll to the type-C chlorotic areas that had already appeared but prevented any further development of this type of chlorosis,

which clearly shows that a deficiency of manganese was the cause of this type of chlorosis and that manganese, rather than zinc, boron, copper, or arsenic, was the corrective agent both in the greenhouse cultures and those conducted in the field in 1931. The evidence also shows that 0.5 p.p.m. of manganese was insufficient to prevent chlorosis entirely but that 1.0 p.p.m. was adequate.

It will be recalled that the excess-calcium and the nitrogen-deficient cultures of the 1930 experiment were free from chlorosis, even though no minor elements were added. As already pointed out, these cultures were the only ones that received calcium chloride; hence, there is a possibility that the calcium chloride might have introduced small quantities of certain minor elements into these cultures and thus prevented the development of chlorosis. The manufacturer's analysis, which accompanied the calcium chloride, was as follows: "Mg and alkalies, 0.20%; BaO, – .001%; Na, trace; Fe, – .001%; SO_3, .01%." In the term "Mg and alkalies," traces of such metals as magnesium, potassium, strontium, and lithium might reasonably be expected to occur. It is doubtful, however, that any appreciable amounts of manganese would be present.

It is unlikely also that the calcium chloride had any effect on the availability of other materials by changing the reaction of the nutrient medium, because the calcium chloride stock solution was practically neutral in reaction and its addition in any amount should have had no effect on the reaction of the nutrient solution. The cultures that were free from chlorosis and also those receiving all elements in medium amounts were tested for reaction and found to have pH values of either 4.6 or 4.7. The solutions, therefore, were sufficiently acid to make available such materials as manganese, aluminum, iron, etc., had they been present. This precludes the likelihood of a lime-induced chlorosis, especially since chlorosis was absent in the excess-calcium cultures. It is unlikely also that the chlorosis resulted from the high acidity making available such quantities of manganese, aluminum, and iron as to be toxic, for these elements were not applied to the sand and, therefore, were not present in toxic amounts, even if totally available.

Sterility and dwarfing: As stated above, the tassels produced by the plants grown in sand cultures in the field in 1930 were mostly sterile (Fig. 4, B, C). These cultures, it will be recalled, received no minor elements. The plants grown in the greenhouse tests in sand that received no minor elements also produced sterile tassels (Fig. 5, A). However, the plants grown in soil (Fig. 5, C) and those grown in sand to which the minor elements were added (Fig. 5, B) produced fertile tassels. These observations indicate that one or more of the five minor elments added functioned

in the development of fertile staminate flowers in these plants. This was confirmed by the fact that there was no evidence of sterility in any of the 1931 cultures. All of these cultures received minor elements and developed functional flowers in both tassels and shoots. Furthermore, the 1931 cultures showed no evidence of the types of dwarfing (Fig. 4) found in the 1930 cultures. The general height of the plants grown in 1930 was only 40 to 50 in., whereas, in 1931, most of the plants ranged between 80 and 100 in. in height.

The studies of other investigators indicate that all of the sterility and much of the dwarfing observed in the 1930 cultures were due to a lack of boron. Thus, Warington (12) found that a deficiency of boron caused death of the growing apices in the broad bean. Johnston and Dore (4) report that the terminal shoots of tomato die when boron is excluded from the growing medium, and Swanback (11) states that one of the most apparent symptoms of boron deficiency in tobacco is the death of the terminal bud. There can be little doubt, however, that the lack of other minor elements also had some effect in reducing the general growth of the plants.

RESISTANCE TO FROST

In connection with the greenhouse tests an interesting observation was made on the effect of the minor elements on the ability of the plants to resist injury from frost. In starting the greenhouse tests to determine the effect of minor elements on chlorophyll development, 6 cans filled with sand were planted with 12 kernels each. When it was found later that this procedure furnished more plants than were needed for transplanting in the chlorosis studies, 3 cans were placed outside the greenhouse and allowed to remain there. One of these received an application of minor elements on September 22 (age, about 3 weeks) and, again, on September 26, in addition to the basal nutrients. A second can received but one application of minor elements, in addition to the basal nutrients, on September 26. The third can received only the basal nutrients and, therefore, served as a check on the effects of the minor elements.

During the night of September 30 there was a heavy frost, the temperature dropping to 34° F. The following day it became apparent that the minor elements had increased the resistance of the young plants to injury from frost. The cans were then moved into the greenhouse to prevent further injury from cold and were supplied with basal nutrients for 3 weeks more. They were photographed on October 20 and are reproduced in figure 7. The plants that received basal nutrients only proved to be very susceptible to frost and were severely injured (Fig. 7, A). The leaves were almost entirely killed and the stalks made little growth during the

FIG. 7. Plants showing effect of additions of minor elements on resistance to injury from frost. A. Received basal nutrients only. B. Received one application of minor elements in addition to basal nutrients. C. Received two applications of minor elements in addition to basal nutrients.

3-week period following the frost. The plants that received only one application of minor elements were but slightly injured (Fig. 7, B). The tips of some of the leaves were killed, but the injury was not severe enough to stunt the plants much during subsequent growth. The plants that received two applications of minor elements showed no evidence of injury from the frost (Fig. 7, C) and made more growth than any of the other cultures. It is very evident from these results that the addition of minor elements enabled the young corn plants to withstand low temperatures much better than where the basal nutrients only were supplied.

SUMMARY

This paper deals with the description, cause, and prevention of disorders that occurred in corn plants grown in pure quartz sand supplied with basal nutrients only. The disorders consisted of three distinct types of chlorosis in the leaves, sterility of the flowers, and a dwarfing of the plants.

The three types of chlorosis have been designated as A, B, and C. Types A and B appear as continuous chlorotic streaks extending from the base to the tip of the leaves. The distinguishing feature between them is that in type A the streaks have ragged or irregular margins, while those of type B have smooth margins. Type C is very different from either A or B, being characterized by discontinuous streaking produced by chlorotic areas distributed at random in the intervascular tissues. Illustrations of these types of chlorosis are given in the text.

Field studies, isolations, and inoculations showed that the disorders observed were not of hereditary or pathogenic origin.

A number of nutritional relations are considered and discussed. The evidence presented shows that the type-A chlorosis was caused by a deficiency of magnesium, type-B by an excess of sodium, and type-C by a deficiency of manganese. Sterility of the reproductive organs is attributed to a deficiency of boron.

It was found that the addition of manganese, zinc, copper, boron, and arsenic to the basal nutrient solution increased the frost resistance of young corn plants grown in sand cultures.

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LITERATURE CITED

1. BROWN, DUKE D. The effect of different proportions or calcium nitrate and potassium di-hydrogen phosphate on the growth of wheat in sand cultures. Soil Sci. **26**: 411–446. 1928.
2. DULEY, F. L., and M. F. MILLER. The effect of a varying supply of nutrients upon the character and composition of the maize plant at different periods of growth. Mo. Agr. Exp. Sta. Res. Bul. 42. 1921.
3. GILBERT, BASIL E., FORMAN T. MCLEAN, and LEO J. HARDIN. The relation of manganese and iron to a lime-induced chlorosis. Soil Sci. **22**: 437–446. 1926.
4. JOHNSTON, EARL S., and W. H. DORE. The influence of boron on the chemical composition and growth of the tomato plant. Plant Physiol. **4**: 31–62. 1929.
5. JONES, J. P. Deficiency of magnesium the cause of a chlorosis in corn. Jour. Agr. Res. **39**: 873–892. 1929.
6. KOEHLER, B., and J. R. HOLBERT. Corn diseases in Illinois. Ill. Agr. Exp. Sta. Bul. 354. 1930.
7. LEE, H. A., and J. S. MCHARGUE. The effect of a manganese deficiency on the sugar cane plant and its relationship to Pahala blight of sugar cane. Phytopath. **18**: 775–786. 1928.
8. MCHARGUE, J. S. Effect of different concentrations of manganese sulfate on the growth of plants in acid and neutral soils and the necessity of manganese as a plant nutrient. Jour. Agr. Res. **24**: 781–794. 1923.
9. ———. Manganese and plant growth. Indus. and Engin. Chem. **18**: 172–175. 1926.
10. SKINNER, J. J., and R. W. RUPRECHT. Fertilizer experiments with truck crops. Fla. Agr. Exp. Sta. Bul. 218. 1930.
11. SWANBACK, T. R. The effect of boric acid on the growth of tobacco plants in nutrient solutions. Plant Physiol. **2**: 475–486. 1927.
12. WARINGTON, KATHERINE. The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. **37**: 629–672. 1923.

CHLOROSES OF THE ROSE

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In the fall of 1927 a mosaic-like disease of roses growing under glass was observed in New Jersey, and since that time various surveys have shown this disease to be widespread throughout the United States. The symptoms of rose mosaic are very variable, depend on environmental conditions for expression, and have been reported as not clearly distinguishable from chloroses due to environmental effects and insect injuries. As a result, considerable confusion and doubt have arisen in regard to the nature of this disease.

It is the purpose of this paper to compare various types of chloroses of the rose and to present results of further experiments on the artificial transfer of rose mosaic to species and varieties of rose used as understock.

LITERATURE REVIEW

In 1909, 1910, and 1914 reports were received by the Plant Disease Survey from J. B. S. Norton in Maryland[1] of a mosaic-like mottling of rose. Norton was inclined to attribute these symptoms, however, to environmental conditions, especially excess soil moisture. Taubenhaus, in 1923[2] and 1925,[3] reported from Texas a condition of roses resembling mosaic. That this was a type of the disorder distinct from lime chlorosis is evident since he also reports the latter type of chlorophyll deficiency. Clinton,[1] in 1923, reported a disease of roses, resembling mosaic, on the Marchioness of Dufferin variety growing in Connecticut.

Ramsbottom (8) describes a chlorosis of rose due to excess lime in the soil. This type of chlorosis was corrected by adding 1 ounce of sulphate of iron crystals to the soil about each plant. Spraying with an iron sulphate solution, 1 oz. to 6 gal. of water, often turned the leaves green. He also stated that the stems showed yellow patches when suffering from this type of chlorosis.

The first description of rose mosaic, originally called an infectious chlorosis (10), listed three Hybrid Tea varieties as being naturally infected, and successful experiments were reported in transferring the disease to healthy plants of a fourth Hybrid Tea variety by means of buds. In the

[1] Letter dated May 12, 1928, to the writer from Dr. R. J. Haskell, formerly in charge of Plant Disease Survey, U. S. Department of Agriculture, Washington, D. C.

[2] Taubenhaus, J. J. (Rose mosaic). U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Suppl. 29: 447. 1923.

[3] ———. (Rose mosaic). U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Suppl. 42: 361. 1925.

following year this trouble was reported to be widely distributed in greenhouses of the Eastern and Midwestern States and Eastern Canada on many varieties of Hybrid Tea roses and on domestic *Rosa manetti* stock (11). It was further reported that the disease had been transferred by means of buds to *R. multiflora* and *R. odorata* but that attempts to transfer it to healthy roses by means of insects failed.

Nelson (7) reported this disease on Pernetiana and Hybrid Pernetiana roses in Michigan and heavy losses on Hybrid Tea varieties. He further reported that, as a result of a survey in California, from 10 per cent to 100 per cent infection was found on plantings of *Rosa manetti* and Ragged Robin understocks. He obtained successful transfer by means of buds and grafts but negative results with insects. White (12) reports it on 25 varieties of Hybrid Tea roses from 8 States and Eastern Canada. He was unsuccessful in transferring the disease to *R. odorata* and to Pernetiana and Polyantha types of roses in limited trials, although he had previously reported successful transfer to *R. odorata*. The apparent recovery of *R. odorata* plants to which the disease had previously been transferred led to some doubt as to the success of the transfer. However, in the light of subsequent experience, this apparent recovery is not unusual.

White (13) gives an extended account of the disease and reports it from 12 States and the Province of Quebec on 27 varieties of Hybrid Tea roses and 2 rose species. In a later article (14) control of the disease is suggested possible by thoroughly roguing the fields of diseased understock, combined with rigid inspection of mother plants from which buds or scions are to be taken. Two cases are mentioned in which it is indicated that some imported *Rosa manetti* plants were infected.

Milbrath (4, 5, 6) reports that surveys in California of roses in greenhouses, fields, and nurseries failed to show any evidence of this disease on *Rosa manetti* and Ragged Robin roses, extensively grown and used as understocks, although it was found on several varieties. His illustrations clearly show that it was identical to the trouble in eastern greenhouses. A quite distinct type of chlorosis, called "albication," was noted on the variety Souvenir de Cladius Pernet. His survey in eastern greenhouses showed the disease to be very prevalent, and he suggests that it might be attributed to a genetic weakness of varieties that have arisen through breeding or as sports from Ophelia. He makes this suggestion in spite of successful transmission experiments (7, 10, 11, 12) and the finding of the trouble on varieties and species of rose with no genetic relationship whatever to Ophelia. It is still more difficult to understand Milbrath's statements in light of a survey conducted by Weiss and McWhorter[4] on the Pacific Coast, in-

[4] Weiss, F., and F. P. McWhorter. Pacific coast survey for rose mosaic. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. 14: 203–205. 1930.

cluding California. This survey reports fields of *R. odorata* in which at least 5 per cent of the plants showed marked and unmistakable symptoms. In addition, several varieties of Hybrid Perpetual roses, as well as 1 Hybrid Wichuriana, were reported infected. No evidences of the disease were found on Ragged Robin stocks in California, contrary to the findings of Nelson, in 1929. Weiss and McWhorter state that it is conceivable that the symptoms were masked at the time of their survey. *Rosa multiflora*, in California, was found to show appreciable percentages of this disease in some fields. Little mention is made of their findings on *R. manetti*, in spite of their expressed purpose of determining the distribution of the disease on rose understocks being grown on the Pacific Coast, and in spite of its primary importance as an understock for greenhouse forcing of roses. The disease was not found on stocks of Texas Wax rose in Texas.

McWhorter[5] describes 2 cases in Oregon, 1 of which indicates that *Rosa manetti* plants became infected naturally in the field from certain possible wild hosts. The other case described indicated that 1 bundle of imported English *manetti* cuttings were 100 per cent diseased. Nineteen other bundles, presumably from the same source, were 100 per cent healthy. Such a case leads to some doubt as to the homogeneity of the 20 bundles.

From this survey of the literature it is evident that the trouble known as infectious chlorosis or mosaic of the rose is widely distributed in the United States, being present in fields of understock on the Pacific Coast, as well as in eastern greenhouses, occurs on many varieties of Hybrid Tea, Hybrid Perpetual, Pernetiana, Hybrid Pernetiana, and Hybrid Wichuriana roses, as well as on the species *Rosa manetti, R. multiflora,* and *R. odorata,* and the variety Gloire des Rosomanes, or Ragged Robin, all used as understock.

During July, 1931, a rather extensive survey of rose stock and rose fields was made in France, Holland, and England. The only cases of a trouble of roses similar to mosaic as it occurs in the United States were found on certain varieties of climbing roses at Kew Gardens, London, England. (Fig. 5, B, C, D.) However, the outstanding symptom on these plants was a veinal type of chlorosis. It was accompanied in some cases with a slight speckling of the foliage. No evidences of similar symptoms were found in any fields of *Rosa manetti* nor on any varieties of roses in any of the 3 countries visited, which, combined, supply the trade with the greater bulk of importations of this item. This survey does not prove that the disease does not exist in these countries, for the symptoms might well have been masked at the time the survey was made.

[5] McWhorter, F. P. Further report on rose mosaic in Oregon. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. 15: 1–3. 1931.

DESCRIPTION OF CHLOROSES

Mosaic. The symptoms of mosaic as originally described on Hybrid Tea roses (10) and later amplified are as follows: The plants are dwarfed, the degree of dwarfing depending upon the variety, severity of infection, and environmental conditions. The dwarfing is expressed in all parts of the plant, including the roots. Buds are often imperfect, on short stems, and bleached. On seriously infected Madame Butterfly plants the blooms, instead of showing the normal light pink petals, tinted with gold at the base, are almost white. The leaves are variously distorted, with the midrib frequently bent and twisted (Fig. 1, B). The leaflets show distinct chlorotic areas, especially along the midrib, which cause the leaflets to pucker and ruffle (Fig. 1, B). The clearing of the veins is frequently pronounced (Fig. 1, A). Usually all leaflets on a leaf show symptoms, but one frequently finds one leaflet of normal size and without symptoms, while the rest of the leaflets show pronounced symptoms. When the chlorosis is confined to one side of a leaflet, distortion occurs, due to unequal growth of the two halves.

This description was based largely upon the appearance of plants of Madame Butterfly variety and has been illustrated in several articles on the subject (10, 13, 14). By experimentally transferring the disease to several rose species a variety of symptoms, previously suspected to be mosaic, have been proved to be expressions of this disease.

Milbrath (4, 5, 6) pointed out the wide variation in symptoms he had observed on different varieties. They ranged from minute speckling and occasional distortion to conditions approximating the above-described symptoms. Weiss and McWhorter[6] also point out that the above symptoms do not always hold on types of roses grown as understock nor on some Hybrid Perpetual roses. They describe the symptoms on *Rosa manetti* as numerous, minute, chlorotic areas, distributed over the entire leaflet or concentrated toward the tip or periphery of the leaflet, with some of the chlorotic specks apparently interveinal.

Two distinct types of symptoms have been observed on *Rosa manetti.* One corresponds to that described for this species by Weiss and McWhorter[7] (Fig. 1, D), except that there is little tendency for the chlorotic areas to be localized in any one portion of the leaflet. The other type of symptom is a more general chlorosis, giving a typical mosaic-like mottled appearance similar to cases of mild mosaic of potato with a slightly more pronounced pattern when viewed by transmitted light (Fig. 1, E). The first type of symptom has been observed on field-grown plants, while the latter type occurred on greenhouse-grown plants. Gradations between these two types of symptoms have been observed.

[6] *Loc. cit.*

[7] *Loc. cit.*

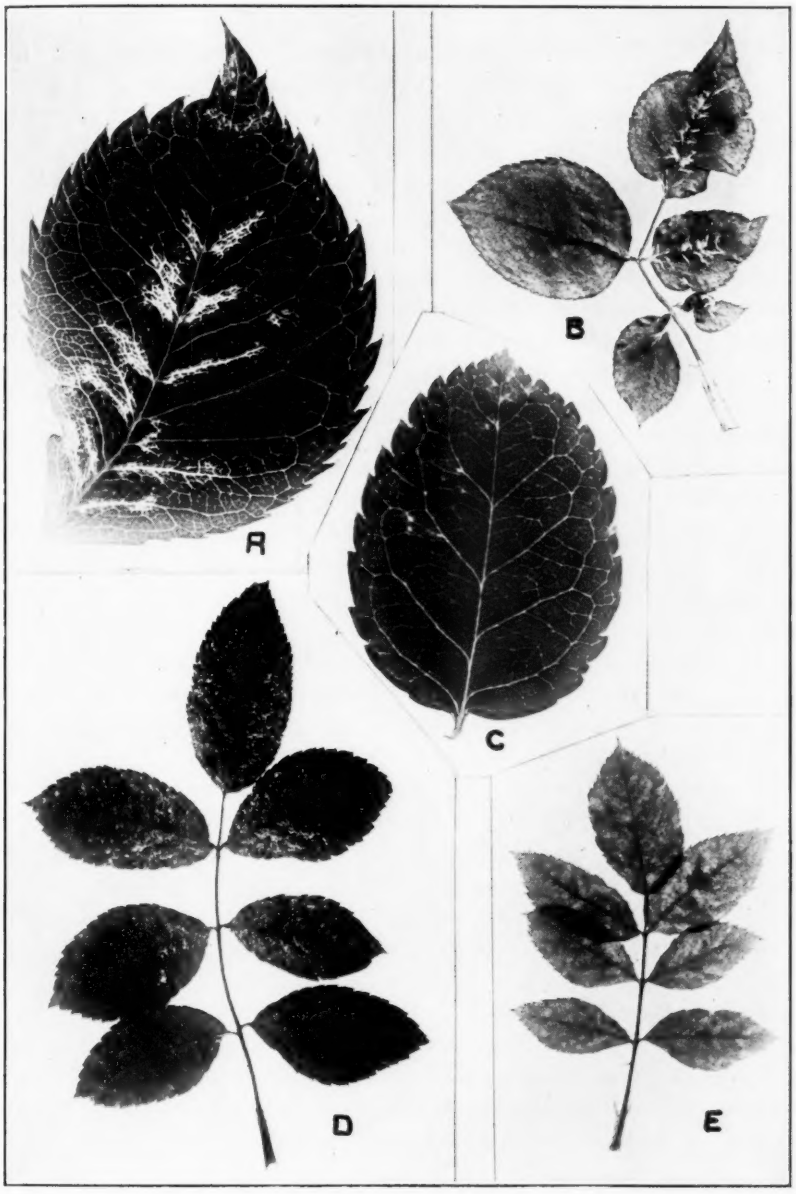

FIG. 1. A. Leaflet of Madame Butterfly variety ×4 showing clearing of veins. Natural infection. B. Leaf of Premier variety showing distortion of midrib and typical mosaic symptoms on leaflets. One leaflet apparently normal. Natural infection. C. Leaflet of Gloire des Rosomanes variety ×2 showing speckling of foliage. Experimentally produced. D, E. Two types of symptoms on *Rosa manetti*. Natural infections.

The disease has not been observed by the writer on naturally infected plants of *Rosa odorata*. It has been transferred to this species under greenhouse conditions by using buds of the variety Madame Butterfly, taken from the bases of leaves showing typical symptoms for this variety, as described above. These buds were kept in a dormant condition. There was apparently no dwarfing of the *R. odorata* canes, and the symptoms, although typical and unmistakable, were slight. These plants were then cut back to within 6 or 8 in. of the crown, which forced the dormant buds of Madame Butterfly into growth. The shoots resulting from these Butterfly buds were all diseased and showed symptoms typical of this variety. The plants of *R. odorata* that became diseased reacted somewhat differently. Strong, vigorous shoots developed, the lower 2 feet of which showed a very marked dwarfing and distortion of the leaves and leaflets (Fig. 2, A) with typical symptoms showing upon them (Fig. 2, D). The canes in this region were also markedly mottled. A similar dwarfing of the foliage has been produced by heavy aphid infestation, but the dwarfed leaflets in such cases never showed chlorotic areas. With further growth these symptoms failed to appear and the leaves were of normal size. A third period of growth then ensued in which the leaf symptoms were again evident, although the leaves were not dwarfed.

On the older leaves of these plants another type of symptom appeared (Fig. 2, G), previously noted on naturally infected plants of Madame Butterfly and Scott's Red Premier (Fig. 4, E) and thought by some to be due to cyanide injury. These symptoms are most evident on the upper surface of the leaflets, as an irregular band of yellowish green between the midrib and the periphery of the leaflet. Frequently, the irregular leaf pattern is lost and the leaf will be blotched in definite areas, sometimes near the periphery, sometimes near the base. These symptoms are not accompanied by dwarfing or malformation of the leaflets, and appear only on the older leaves, which have reached maturity. The symptoms illustrated in figure 2, G, on *Rosa odorata* were produced on plants that had never been subjected to cyanide fumigation.

On *Rosa multiflora* a condition similar to that on *R. odorata* occurred. As long as the diseased buds of Madame Butterfly remained dormant the symptoms on the *multiflora* plant were slight. When these plants were cut back and the diseased buds were forced into activity, and strong shoots of the *multiflora* stock were forced to break from the bases of the older canes, the symptoms became more pronounced. In all cases the diseased Butterfly buds produced diseased shoots showing symptoms typical of the variety. The lower portions of the *multiflora* canes showed decidedly dwarfed and malformed leaves but no other leaf symptoms. The canes in these areas were also mottled but not so strongly as the *odorata* canes. Subsequent

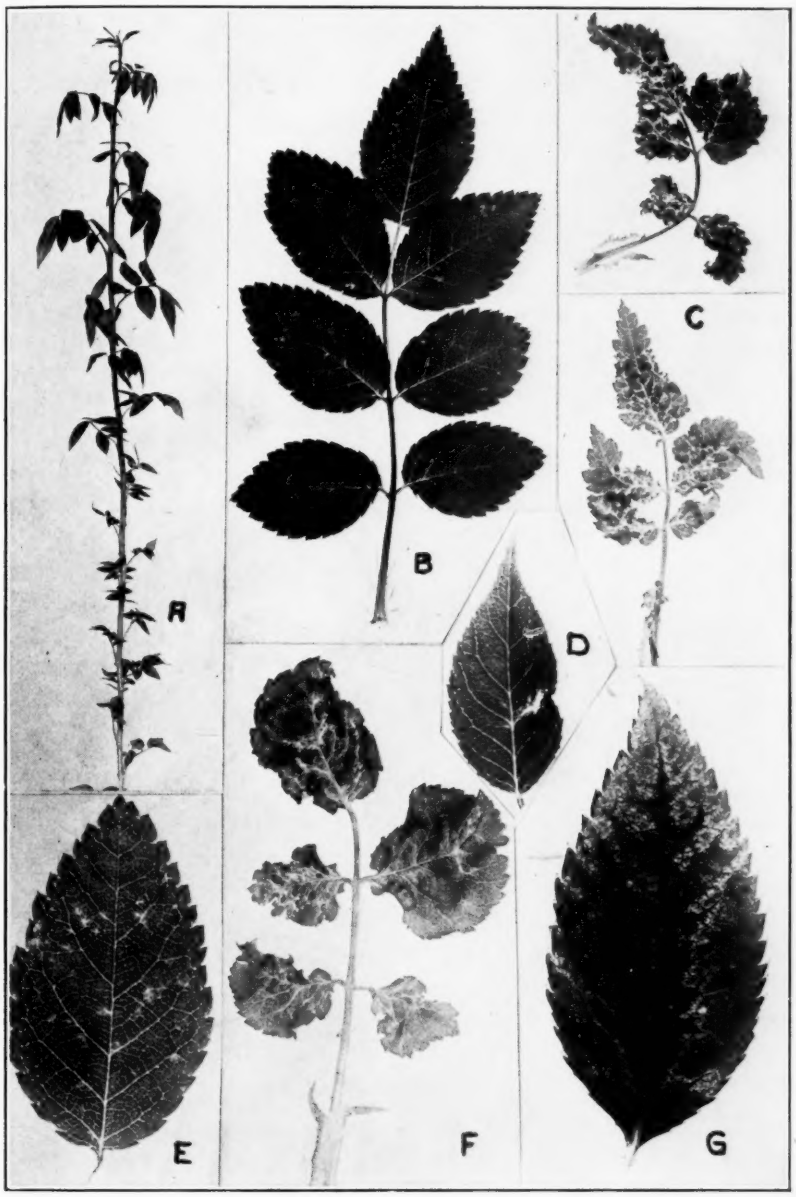

FIG. 2. A. Cane of *Rosa odorata* ×1/12 showing dwarfing and malformation of lower leaves. Experimentally produced. B. Leaf of *R. multiflora* showing speckling. Experimentally produced. C. Two leaves of Texas Wax rose showing pronounced symptoms of mosaic. Natural infections. D. Leaf of *R. odorata* ×2 showing typical symptoms. Experimentally produced. E. Leaflet of *R. multiflora* ×2 showing speckling. Experimentally produced. F. Leaf of American Beauty budded on Texas Wax, showing mosaic. G. Leaflet of *R. odorata* ×2 showing symptoms of mosaic experimentally produced.

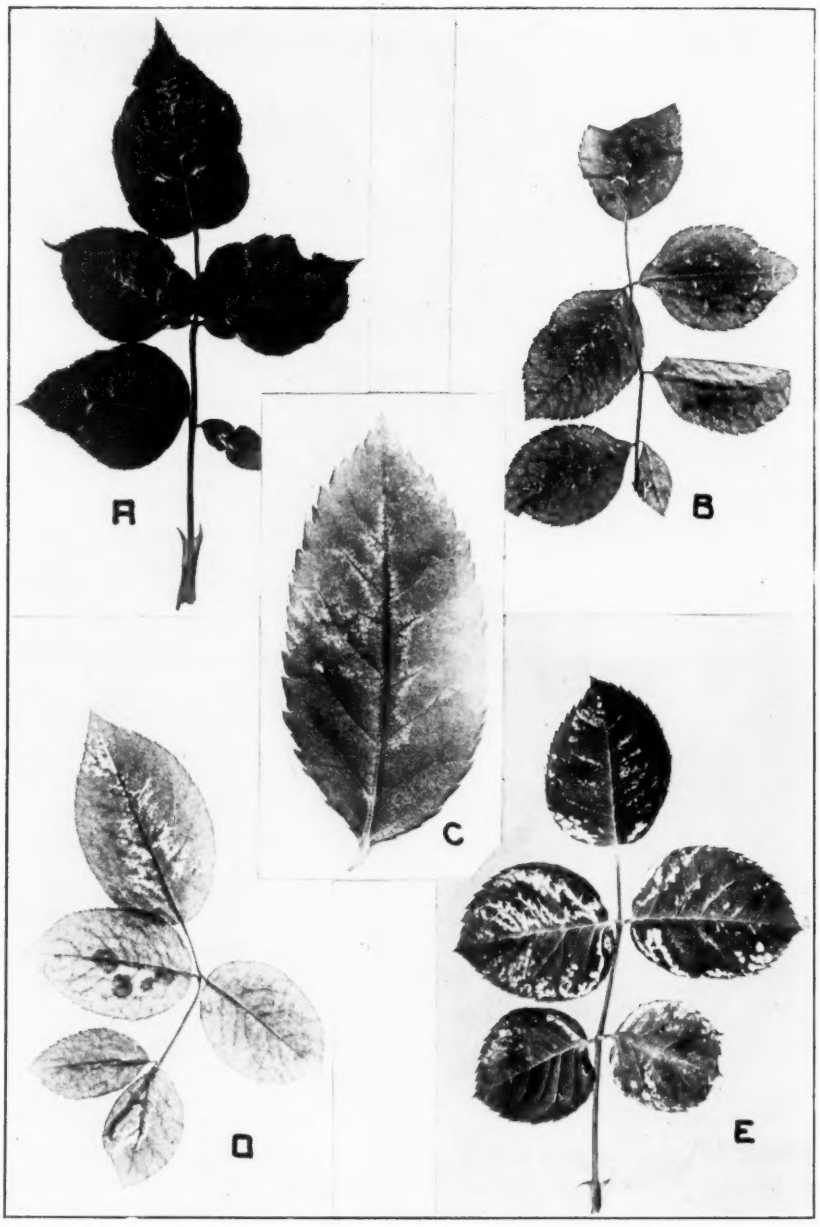

FIG. 3. A. Leaf of Premier variety with typical mosaic symptoms. B. Symptoms of mosaic on leaf of La France variety. C. Leaflet of *Rosa odorata* × 2 with symptoms of mosaic experimentally produced. D. Symptoms of mosaic on Duchess of Wellington variety. E. Albication of Golden Ophelia variety.

growth was apparently normal, but there then ensued a period, corresponding to that of the *odorata* stock, in which the leaves showed a decided speckling (Fig. 2, B, E). Previous experiments on the thorny variety of *multiflora* produced symptoms of a more mottled type, midway between the speckling and the symptoms described for Madame Butterfly. Both types of *multiflora* are evidently susceptible to this disease. No evident decrease in vigor of the *R. multiflora* stock was noticeable in either case, under greenhouse conditions.

On the Gloire de Rosomanes variety, or Ragged Robin, widely used as understock, only the speckling type of symptom appeared. The leaves were not malformed in any way, vigor was not impaired, and dwarfing did not result (Fig. 1, C).

The symptoms observed on Texas Wax rose,[8] extensively used as an understock in Texas, occurred on naturally infected plants obtained from Texas and grown under greenhouse conditions. The leaves were decidedly dwarfed, distorted, and malformed. The leaflets also were extremely dwarfed and puckered and showed a very marked chlorosis, limited largely to the veins (Fig. 2, C). Some speckling of the foliage also was present, in the interveinal areas. American Beauty roses, budded on infected Texas Wax plants, showed similar symptoms (Fig. 2, F).

That the symptoms of this disease vary with the variety has been indicated by Weiss and McWhorter.[9] Milbrath (4, 5, 6) mentions a trouble on two Souvenir de Cladius Pernet plants that he termed albication, in which the leaves developed white patches or streaks. Figure 3, A to E, illustrates a transition from symptoms generally recognized as being associated with mosaic to those that Milbrath has termed albication. The writer has observed symptoms as expressed by both Premier (Fig. 3, A) and Golden Ophelia (Fig. 3, E) on the same plant. He also has reproduced the symptoms as expressed on the Duchess of Wellington variety (Fig. 3, D) by using buds from Madame Butterfly plants showing typical symptoms of mosaic, as described for this variety. Attempts to transfer the condition of Golden Ophelia (Fig. 3, E) to healthy plants failed because the buds used failed to unite.

NUTRITIONAL CHLOROSES

Chloroses of foliage due to deficiencies or excesses of certain soil constituents are not uncommon and have been attributed either to a lack of available magnesium, manganese, nitrogen, and iron or to excesses of nitrogen, sulphur, manganese, and calcium. Excesses of calcium increase the alkalinity of the soil to a point at which the solubility and thus the avail-

[8] *Rosa multiflora* × *R. Chinensis.*

[9] *Loc. cit.*

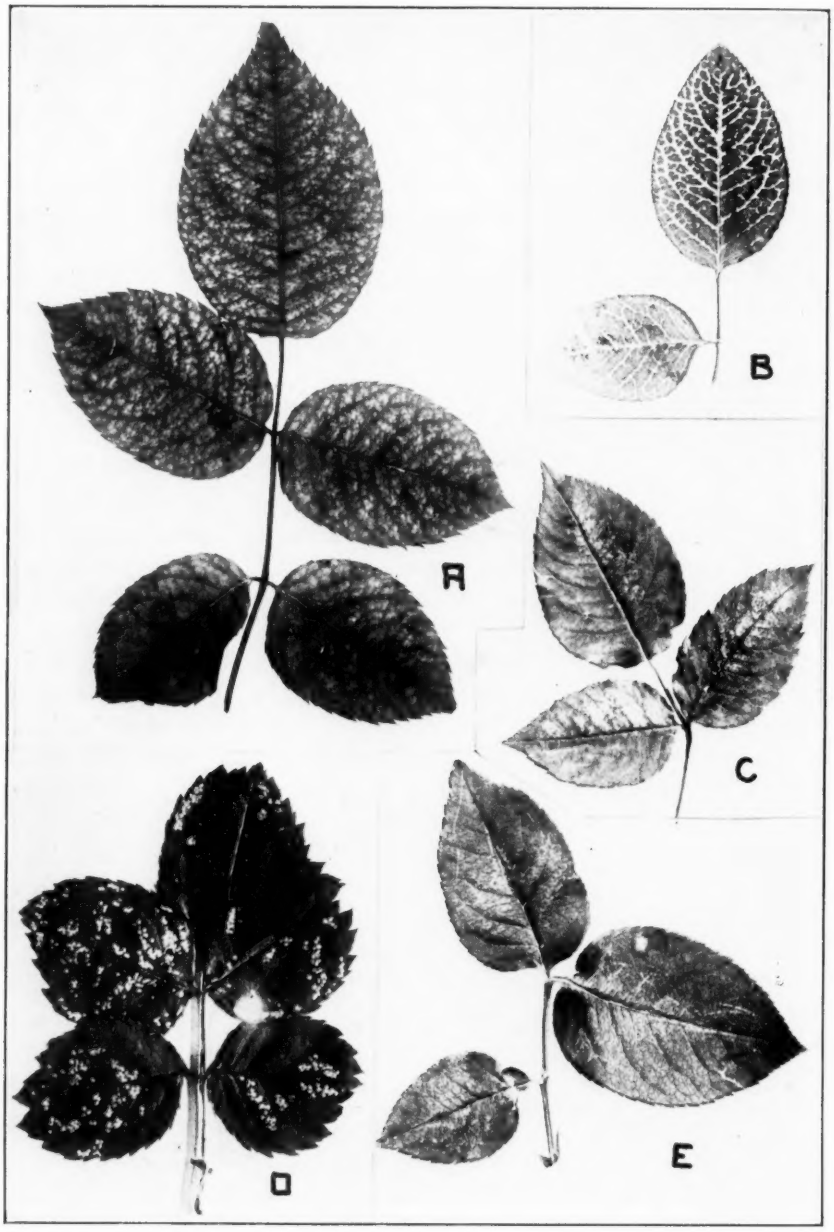

FIG. 4. A. Iron chlorosis on Premier variety. B. Veinal chlorosis on Premier variety. C. Red-spider injury on Scott's Red Premier variety. D. Leaf-hopper injury on Madame Butterfly variety. E. Symptoms of mosaic on Scott's Red Premier variety. Natural infection.

ability of certain elements essential to chlorophyll production, are greatly diminished. Chloroses due to such unbalanced soil conditions, with the exception of nitrogen deficiencies, are expressed as interveinal chloroses, and, as the condition becomes severe, the entire interveinal area becomes devoid of chlorophyll and yellow. The last places in the leaves to lose chlorophyll are about the larger veins. Deficiencies of nitrogen are expressed as a uniform yellowing of the entire leaf.

Lime chlorosis is not uncommon on roses grown in eastern greenhouses, appearing more prevalent and serious in the spring than at any other time of the year. It is frequently due to excess alkalinity of the soils in which the plants are growing. That it may also be caused by other factors is evident, for roses growing in soil with an acidity of pH 5.5 to 6.0 have been seen showing interveinal chlorosis, commonly called iron or lime chlorosis (Fig. 4, A).

Such types of chloroses cannot be mistaken for mosaic. The light yellow-green areas occur regularly scattered over the entire leaflet, are at first faint, but become increasingly more pronounced, and are not accompanied by any type of leaf malformation. In severe cases, the new growth may appear yellow and older leaves may fall. The variety Souvenir de Cladius Pernet is especially subject to this chlorosis when grown under glass. The condition is correctable. Ramsbottom (8) reports that iron sulphate, worked into the soil or spraying the plants with iron sulphate solutions has brought about recovery. The writer's experience has been that the use of soil-acidifying materials will bring about recovery in those cases that are due to excess alkalinity of the soil. Such methods have no effect on the elimination of the symptoms of mosaic.

This condition also is not to be confused with etiolation. Milbrath (4, 5, 6) states that cases of mosaic are difficult to separate from cases of etiolation. Etiolation is a term denoting loss of chlorophyll in leaves combined with an increase in the distance between nodes. It is brought about by growing plants in darkness and can be mildly simulated by decreased light intensities. There is no lengthening of the nodes of rose plants infected with mosaic; in fact, the reverse is often true. The loss of chlorophyll in etiolated plants is uniform over the entire leaflet, contrary to the manner of chlorophyll loss in leaves of mosaic-infected plants, which occurs in isolated areas surrounded by tissue of normal color. Furthermore, mosaic-infected plants exhibit chlorosis in full sunlight, which would not result if the symptoms described for mosaic were due to light deficiencies.

VEINAL CHLOROSIS

Occasionally there is found a plant some of whose leaves show a marked veinal chlorosis (Fig. 4, B). The only cases observed were in a house of

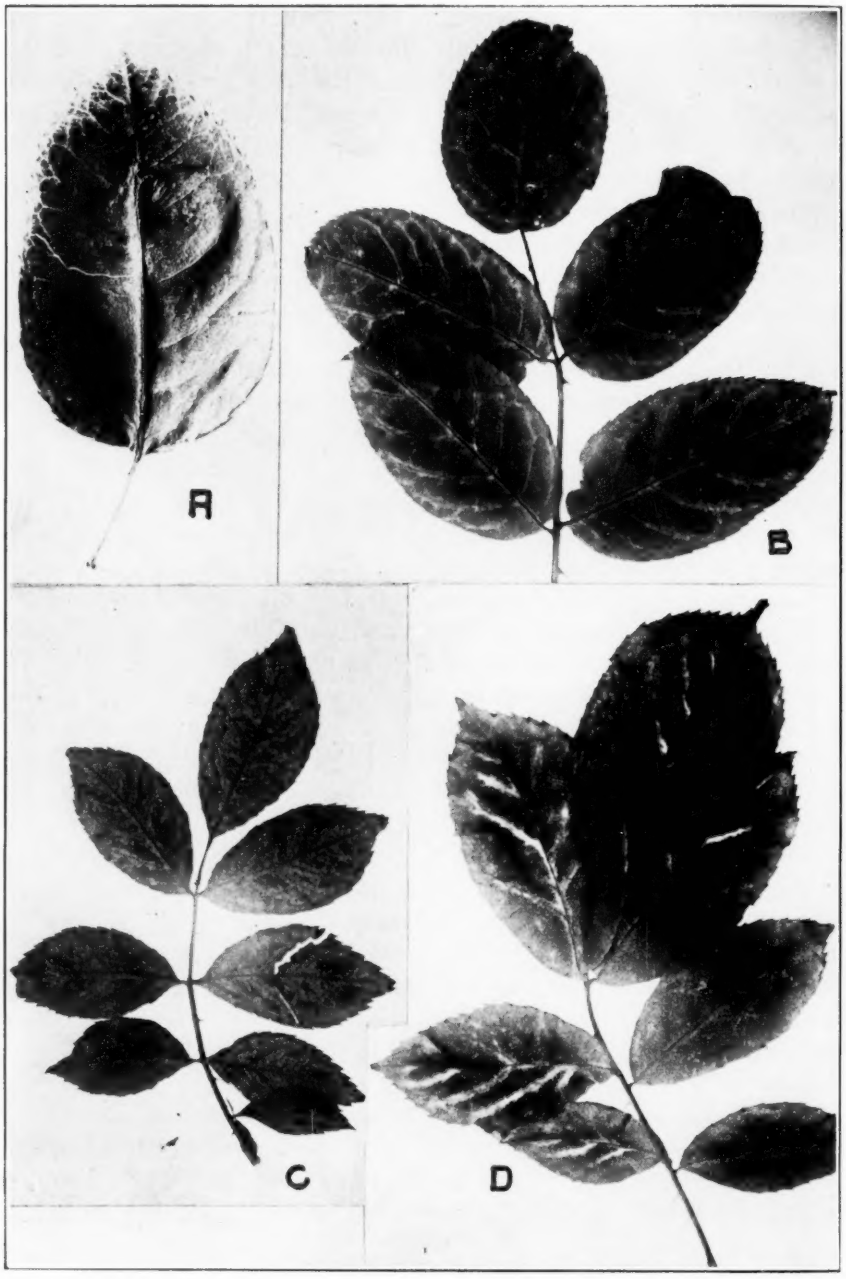

FIG. 5. A. Cyanide injury on Talisman variety. B. Veinal chlorosis on Multiflora variety Helene. C. Veinal chlorosis on Hybrid Wichuriana variety Sander's White. D. Veinal chlorosis on Multiflora variety Ariel.

Premier variety, which showed 35 per cent of the plants with typical mosaic symptoms, and a few roses in Kew Gardens, London, England. The few plants observed showed only the veinal type of chlorosis, and its relation, if any, to mosaic is not known. The leaves were not distorted in any way and the plants were not visibly dwarfed. Such distinct limitations of the chlorosis to the veins has not been observed in any of the plants to which mosaic has been experimentally transferred. The similarity of symptoms, however, as expressed by the variety Ariel (Fig. 5, D), to those illustrated by Rawlins and Horne (9) for a graft-infectious disease of the cherry is striking, with the exception of the color reaction expressed by the two hosts.

INSECT CHLOROSES

It has been the opinion of some that the condition referred to as an infectious chlorosis or mosaic was but the result of direct insect injury,[10] and many investigators, unfamiliar with the disease, are inclined to this view.

Milbrath (6, 7, 8) reports that some of the symptoms he observed in a survey of eastern greenhouses bore a strong similarity to injuries caused by various agencies, such as thrips, red spiders, and aphids, all of which, he said, were primary pests on roses. There are only two such pests on roses in eastern greenhouses, one a mite (red spider) and the other an insect (aphid). Thrips, in eastern greenhouses devoted to rose forcing, are not a primary pest, and attempts to colonize them on roses failed under winter greenhouse conditions. The rose midge has been present in isolated houses but is not commonly encountered. The rose midge injures by chewing. It feeds upon the young growing tips and undeveloped buds and causes malformed leaves, injured blooms, and a stunted condition or death of the terminal growth. No chlorosis of the foliage is produced and mosaic, therefore, cannot be attributed to the presence of this pest.

Leafhoppers are not common on roses under glass, and attempts to colonize them on greenhouse roses during the winter months have met with little success. Their presence results in a loss of chlorophyll at the point of feeding, causing a type of injury so distinct from any of the symptoms produced by mosaic that the two cannot be confused (Fig. 4, D). Furthermore, mosaic may occur in the absence of leafhoppers and it is therefore evident that this insect pest cannot be the direct cause of this disease.

Red spiders are very common on greenhouse roses. They colonize on the under surface of the leaflets, causing injuries very similar to those produced by the leafhopper, except that the individual feeding points are much smaller. On the upper surface of the leaves, above these colonies,

[10] Johnson, K. M. S. Conference on importation of fruit and rose stocks. Fed. Hort. Board. Mimeog. Rpt., p. 51, June 27, 1928. Washington, D. C.

mottled greenish gray areas appear (Fig. 4, C). The chlorosis produced is quite dissimilar to the yellowish or yellowish green produced by mosaic. Furthermore, the spider, in various stages, from the egg to the adult, can be found on the under surface of spider-injured leaves. Mosaic symptoms are not correlated in any way with colonies of red spider and have appeared on plants on which no spider has ever been present.

Aphids (the common green fly of the commercial grower) are also common on greenhouse roses, clustering about the growing tip and sucking out plant juices, causing a stunting of growth and, if abundant, distortion of foliage. Aphids, feeding on young foliage before it unfolds, frequently produce on that foliage a scattered yellow speckling. This might be mistaken for mosaic, if no other symptom of the disease are present, especially if the speckling is observed after the aphids have migrated to other plants. It usually is not difficult, however, to determine whether the speckling is due to aphids or to mosaic. Aphids never cause the pronounced symptoms typical of mosaic. Furthermore, they have never been seen in sufficient numbers to account for the effects of mosaic in commercial rose houses where periodic fumigation is practiced and where mosaic is general.

In the fall of 1929, 6 mosaic-infected plants of Madame Butterfly were brought from the field to the greenhouse, cut back severely, entirely stripped of leaves, and planted in a ground bed in insect-proof cages. Normal growth took place for a period of 8 weeks, at the end of which time a few typical symptoms of mosaic appeared on 1 of the plants. At the end of 11 weeks all 6 plants were showing typical symptoms of mosaic, without the association of an insect of any kind. It is evident that the symptoms expressed by this trouble are not due to direct insect association.

CYANIDE INJURY

Fumigation with cyanide for the control of aphids is a common practice in eastern greenhouses. Excess dosage will produce a chlorosis of rose foliage but a type of chlorosis quite distinct from that produced by mosaic. With cyanide injury the chlorophyll is destroyed around the margins of the leaflets and is more severe and extensive at the tip. The chlorosis extends down the veins (Fig. 5, A) and is a bright yellow to a bleached whitish color. In severe cases of cyanide injury the injured tissue becomes brown and dead. This chlorosis shows up shortly after fumigation and can be diagnosed correctly without difficulty.

DISCUSSION

The possibility of this disease being due to direct insect injuries is precluded by the production of typical mosaic symptoms on foliage of naturally infected plants grown in insect-proof cages, without any insect asso-

ciation. Furthermore, the injuries produced by rose insects are quite distinct from the symptoms of mosaic. Likewise, chlorosis produced by unfavorable soil and atmospheric conditions, and the destruction of chlorophyll by cyanide fumigation, are readily diagnosed and easily distinguished from mosaic.

Milbrath (4, 5, 6) states that the symptoms of mosaic are difficult to separate from types of chloroses due to etiolation, icterus, and albication. The chlorosis due to etiolation is initiated by light deficiencies and is of a uniform character, which will not permit it to be confused with mosaic. Similarly, conditions described as icterus, in which the chlorophyll of plants becomes deficient, are due to unfavorable growing conditions and are non-transmissible and the yellowing of leaves is uniform, in marked contrast to the speckling, mottling, and isolated chlorotic areas due to mosaic infections.

Conditions of albication, in which the leaves or portions of leaves develop whitish or yellowish areas, do approach the pronounced symptoms of mosaic. The term albication does not imply that the condition is not transmissible. In fact, the infectious chloroses with which Baur (1, 2, 3) worked were albications or variegated forms of Ligustrum, Laburnum, Malva, etc. Milbrath, himself, speaks of the albication he found on Souvenir de Cladius Pernet as a "definite instance of a transmissible condition of albication" (5, 6). However, he merely proved by his experiments that the condition was perpetuated by vegetative means. He did not obtain a transmission of the condition to the plant to which he transferred buds of the albicant.

The wide variation in symptoms known to be transmissible and associated with mosaic and the fact that symptoms of both albication and mosaic have been found on the same plants of Golden Ophelia indicate that the albication of the rose may be a pronounced symptom of mosaic. (Fig. 3, A–E). Some plants of the variety Conrad Ferdinand Meyer, growing in the New York Botanical Gardens, which showed pronounced symptoms of albication, were brought to my attention in 1929 by B. O. Dodge. In 1930 these same plants and, in addition, plants of the variety A. K. Williams, not far removed, showed the same symptoms. This same condition has been observed on plants of the Premier and Butterfly varieties growing outdoors, which had been transplanted from the greenhouse and which originally showed only typical mosaic symptoms.

The perpetuation of the symptoms of mosaic has been proved beyond any doubt by numerous budding experiments. Buds from infected plants will always produce infected shoots. Furthermore, in a considerable percentage of all trials, the entire plant, originally healthy, into which diseased buds are inserted, will become diseased. In such cases a transmission of the disease to the budded plant has taken place. These cases do not

constitute merely the perpetuation of a character by vegetative means but prove the transmissibility of the abnormal condition.

Milbrath also points out that "recovery has been very significant in this trouble of the rose" (4, 5, 6). In support of this statement he cites one case in which the disease in a period of 2 months had decreased from 50 per cent to 24 per cent. Another case was cited in which several diseased plants were cut back and started anew and at the end of 4 months were reported as having recovered. Other similar instances are given.

Following severe pruning, growth as a rule takes place normally for varying periods of time. Plants have been grown for 3 years under outdoor conditions, but they have always shown the typical symptoms of mosaic at some time during the growing season. The expression of the symptoms of this disease seems to be particularly sensitive to environmental conditions, and the failure to exhibit typical symptoms is no proof that the plants are recovered. Plants, known to be infected, have been carried from a severe pruning to bloom without any symptoms appearing. A second severe pruning, however, was again followed by new growth and this growth exhibited typical symptoms in due time. Until further studies can be made upon environmental conditions, as related to symptom expression, this reaction will remain unexplained. In no case have I ever seen recovery, meaning absolute freedom of the trouble. Plants may and normally do fail temporarily to show symptoms following heavy cutting back and in response to certain unknown environmental conditions but, eventually, with the return of proper environmental conditions, the symptoms will again be expressed.

Milbrath further states (4, 5, 6) that, if this were a virus disease, it would be systemic. Buds have been taken from canes of infected roses from bases of leaves, some of which were showing symptoms and some of which were not. In cases where the buds used formed a union and grew successful transfer of the disease was accomplished by buds from bases of leaves that were apparently normal and healthy, as well as by buds from bases of leaves showing pronounced symptoms. The failure of some leaves on infected plants to show symptoms cannot be explained by assuming that rose mosaic is nonsystemic. The absence of symptoms on certain leaflets, while other leaflets of the same leaf show pronounced symptoms, is not infrequent. Leaves developed at the same time on different shoots of the same plant or on different plants, under exactly similar external environment, may or may not show symptoms. Such observations would indicate a different internal environment. It has been noted that plants, high in carbohydrates and low in nitrogen, often fail to show symptoms when diseased buds are united with them.

SUMMARY

The symptoms of the infectious chlorosis of rose known as mosaic are given for various varieties and species of rose and compared with other types of chlorosis.

The species *Rosa manetti, R. multiflora, R. odorata,* and the variety Gloire de Rosomanes, or Ragged Robin, as well as the variety known as Texas Wax, all used as understocks, have been found susceptible. The symptoms of mosaic on these species and varieties may be quite distinct from the symptoms expressed by certain Hybrid Tea varieties.

LITERATURE CITED

1. BAUR, E. Ueber die infektiöse Chlorose der Malvaceen. Sitzungsber. Konigl. Preuss. Akad. Wiss. **1906:** 11–29. 1906.
2. ————. Ueber infektiöse Chlorosen bei *Ligustrum, Laburnum, Fraxinus, Sorbus* und *Ptelea.* Ber. Deut. Bot. Ges. **25**: 410–413. 1907.
3. ————. Ueber eine infektiöse Chlorose von *Evonymus japonicus.* Ber. Deut. Bot. Ges. **26:** 711–713. 1908.
4. MILBRATH, D. G. Infectious chlorosis of the rose. Western Flor. **13** (29): 20–30. 1930.
5. ————. A discussion of the reported infectious chlorosis of the rose. Florist's Exchange and Hort. Trade World **74:** 11, 33. July 26, 1930.
6. ————. A discussion of the reported infectious chlorosis of the rose. Mo. Bul. Dept. Agr. Calif. **19:** 8: 535–544. 1930.
7. NELSON, R. Infectious chlorosis of the rose. (Abst.) Phytopath. **20**: 130. 1930.
8. RAMSBOTTOM, J. Rose diseases caused by fungi. The Enemies of the Rose. New ed. Nat. Rose Soc. Westminster, England, pp. 111–165. 1925.
9. RAWLINS, T. E., and W. T. HORNE. "Buckskin," a destructive graft-infectious disease of the cherry. Phytopath. **21**: 331–335. 1931.
10. WHITE, R. P. Diseases of ornamental plants. An infectious chlorosis of rose. N. J. Agr. Exp. Sta. 49th Ann. Rpt. **1927/28**: 278. 1928.
11. ————. Diseases of ornamental plants. N. J. Agr. Exp. Sta. 50th Ann. Rpt. **1928/29**: 42–43. 1929.
12. ————. An infectious chlorosis of rose. (Abst.) Phytopath. **20:** 130. 1930.
13. ————. An infectious chlorosis of rose. Amer. Rose Ann. **15:** 87–90. 1930.
14. ————. Infectious chlorosis of rose. Florist's Exchange and Hort. Trade World **73**: 46. Jan. 25, 1930.

A STUDY OF LABORATORY METHODS FOR INVESTIGATING THE RELATION BETWEEN MOISTURE CONTENT OF WOOD AND FUNGAL GROWTH

FERDINAND W. HAASIS[1]

INTRODUCTION

GENERAL PROCEDURE. This study was conducted in the Forest Products Laboratory at the School of Forestry, University of Idaho, during the years 1928–1930. It constituted one phase of a comprehensive study of the optimal and minimal moisture contents of wood for the growth of decay-producing fungi, under the general supervision of Dr. E. E. Hubert, of the School of Forestry. The chief immediate objective was the development of a relatively simple technique whereby studies of the relation between moisture content and fungal activity might be satisfactorily made. Acknowledgment is made to Doctor Hubert for suggestions and criticisms in connection with both experimental work and presentation of the results.

In these experiments the only host used was sapwood of western yellow pine, *Pinus ponderosa* Dougl. The saprophytic organism used was *Fomes roseus* (Alb. and Schw.) Fr., which caused a brown pocket rot. This species occurs in nature on the wood of western yellow pine (see Anderson, *et al.*[2]), although the strain used was a pure culture isolated by Doctor Hubert, from jack pine, *Pinus divaricata* Du Mont de Cours. The cultures were incubated at a temperature of about 27° C. No study was made of the effects of varying amounts and qualities of light in the culture chamber. Incubation was continued for a period of approximately 6 months, 180 days for the most part.

In experiments of this type the activity of the fungus is considered to be represented by the relative loss in oven-dry weight of the wood. This is a method that has been used to a considerable extent in such studies (*cf.* Humphrey,[3] p. 265, Schmitz,[4] Hubert,[5] pp. 14, 16, 17).

[1] Carnegie Institution of Washington. Formerly, School of Forestry, University of Idaho.

[2] Anderson, P. J., *et al.* Check list of diseases of economic plants in the United States. U. S. Dept. Agr., Dept. Bul. 1366. 1926.

[3] Humphrey, C. J. Decay of lumber and building timbers due to *Poria incrassata* (B. and C.) Burt. Mycol. **15**: 258–276. 1923.

[4] Schmitz, Henry. Studies in wood decay. V. Physiological specialization in *Fomes pinicola* Fr. Amer. Jour. Bot. **12**: 163–177. 1925.

[5] Hubert, E. E. A study of laboratory methods used in testing the relative resistance of wood to decay. Idaho Univ., School of Forestry Bul. 3. 1929.

RESULTS AND DISCUSSIONS

Addition of moisture. At first sticks of wood about 14 cm. long and 1 cm. square were used. These were oven-dried at 100° C. to constant weight and then a small amount of water was added so that about 1 cm. of the lower end of the stick was immersed. It was hoped that the water absorbed by the stick would become so distributed that a moisture-content gradient would result. It was planned, such a gradient having been established, to place some inoculum near the middle of the stick and, after a period of incubation, to study the activity of the fungus at various heights.

It was soon learned, however, that, although the water would creep up the sides of the stick and even over the top, its progress through the stick was much slower. It was evident that the results of moisture determinations in samples from these sticks must be accepted somewhat cautiously because of the irregularity of the moisture distribution. This irregularity, it was found, can be very graphically demonstrated by the use of indelible pencils.

Obviously, in making studies of the relations between moisture content and decay, it is essential to have the water distributed with considerable uniformity through the test material. Accordingly, attempts were made to insure uniform distribution of added water.

In these experiments the length of the test pieces was much reduced, blocks about 2.5 cm. long and 1.3 cm. square being substituted for the sticks. These blocks were weighed individually, the desired moisture content was decided upon, and the water to be added was computed, assuming the air-dry wood to contain the same amount of moisture as check blocks whose moisture content was ascertained. This amount of water was placed in a cotton-plugged test-tube with the block and the preparation autoclaved under various conditions. Individual preparations were, for example, autoclaved at 2-lb. pressure for 45 min., cooled, and autoclaved at 5–7-lb. pressure for 30 min.; autoclaved at 5-lb. pressure for 30 min.; and autoclaved 30 min. at atmospheric pressure, and, 2 days later, for 30 min. at 7-lb. pressure.

The moisture actually absorbed under these conditions seemed to have little relation to the amount intended. Therefore, flat-bottom vials were substituted for test-tubes, and a number of different autoclaving methods were tried; but the moisture distribution, as indicated by indelible-pencil streaks, was not entirely uniform with either cotton plugs or toy-balloon caps. Of the methods tried, autoclaving for 1 hour at atmospheric pressure (98.5° C., as ascertained by a number of observations) seemed to give the best results. Petri-dish autoclaving gave unsatisfactory moisture distribution.

Acting on a suggestion of Doctor Hubert's, Sitka-spruce veneer (0.03 cm. thick) also was given a trial, 5-cm. squares being placed in individual Petri dishes and water added to each dish. It was thought that better distribution might be secured in these thin pieces. It was found, however, that the distribution was even more irregular than in the case of the blocks. It is to be borne in mind that the amount of water added was proportional to the *weight* in both cases, and with the veneer squares was very small, indeed (0.04–0.15 gm.). Condensation on the inside of the Petri dish was very pronounced, and this resulted in spotty moisture distribution in these small pieces. It was observed, also, that such thin sheets may be moist on one side and dry on the other (0.01 in. away.)

The idea suggested itself that, if the blocks were heated when placed in water, the absorption might be more uniform than theretofore. Accordingly, a couple of sets that had been thoroughly warmed by being kept in a 100° (C.) oven 3 days were placed in water in vials and then autoclaved an hour at atmospheric pressure. The moisture distribution in these blocks, however, proved to be not notably uniform.

Stender dishes were used next as containers during the autoclaving. There were available dishes 4 cm. high and 8 cm. in diameter that would conveniently hold 10 of the $1.3 \times 1.3 \times 2.5$ cm. test blocks. These dishes had ground edges and ground grooves in the lids fitting over the edges. The blocks were autoclaved an hour at atmospheric pressure. This method was found eminently satisfactory and was tentatively adopted as standard, the blocks being preferably autoclaved the day after adding the water and left a day after autoclaving, before using.

One comparative test was run using the following containers:

Two blocks lying on a long side in a small stender dish (about 2 by 4 cm.);
Two blocks lying on a long side in individual small stender dishes;
Two blocks in cotton-plugged vials;
Two blocks in cotton-plugged test tubes;
Two blocks lying on a long side, in a deep Petri dish.

In this test the moisture distribution was fairly uniform in one block autoclaved in an individual stender dish, in one block in a vial, and in the two blocks in the Petri dish. In the others it was not very uniform.

The 10 test blocks of a series were habitually weighed individually and water enough added to the lot in the stender dish to give the desired average moisture content. The results were not very precise, the individual blocks of a lot absorbing widely different amounts, although the distribution was uniform in each block. The average moisture content of a lot could, however, be approximated fairly closely. The following tabulation, while including a considerable number of interpolated figures, will give some idea

of the amounts of water required in using this method. The figures apply to western-yellow-pine sapwood blocks of medium grain, approximately $1.3 \times 1.3 \times 2.5$ cm. in dimensions and averaging 6 per cent moisture content.

Average moisture content desired 10 blocks, per cent	Approximate amount of water to be added, ml.
10	1.0
20	3.0
30	5.0
40	7.0
50	9.0
60	11.0
70	13.0
80	15.0
90	17.0
100	18.5
110	20.0
120	21.5
130	23.0
140	24.5
150	26.0

In order to ensure a close approximation to the desired absorption by individual blocks, it would probably be necessary to use a separate dish for each block. Needless to say, the more uniform in texture, specific gravity, and air-dry moisture content the test material is, the more precisely can the desired amount of water be added and the more significant will be comparisons of decay in blocks of diverse moisture content.

Maintenance of uniform moisture content. While a satisfactory method of adding moisture to the test blocks has been developed, the problem of maintaining the moisture content during an incubation period of as much as 6 months has not yet been solved.

Following a method developed by Hubert,[6] culture tubes plugged with cotton were capped with toy balloons. In reweighing such tubes, after from 10 to 40 days in the culture chamber, it was found that they had lost weight at the rate of about 0.05 gm. a day. A study was accordingly made of the amount of water lost during a period of 27 days through a number of different stoppers, two of each type being tested. At the beginning of the experiment 20 ml. of water was added to each of 10 test tubes and the several stoppers then applied. The average losses in weight were as follows:

[6] *Loc. cit.*, p. 38.

	27 days	Per day	Per 10 days
Cotton stoppers	3.79 gm.	0.14 gm.	1.40 gm.
Cotton stoppers, capped with toy balloons	1.30	0.05	0.48
Cork stoppers (No. 12) with transverse lenticels	0.28	0.01	0.10
Paraffined cork stoppers, similar to above	0.04	.....	0.01
Rubber stoppers (No. 5)	0.07	.....	0.03

From these experiments it is evident that the paraffined cork was the most effective stopper tested, in preventing loss of moisture. The plain corks were the most variable, the 27-day losses being 0.44 gm. and 0.12 gm., respectively. The variation in the other pairs was relatively slight.

From this study it appeared that in using the balloon-cap method of covering test-tubes there can be expected a considerable loss of water, though this loss is only about $\frac{1}{3}$ that that occurs without the caps. Yet, this loss might quite appreciably affect the results in studies of the influence of moisture content. It seemed that for such work a tighter stopper would be much preferable; and paraffined corks were, accordingly, tentatively adopted as standard for the cultures started after May, 1929.

There also should be mentioned here a test of sodium silicate as a coating for cork stoppers. For this purpose the corks were dipped in a commercial solution of the material (water glass). This is perhaps easier to apply than hot paraffin. It has, however, certain very pronounced disadvantages. If dried on a glass plate, the treated corks stick much more than do paraffined corks, allowed to cool on a glass plate. Double dipping is advisable in using either water glass or paraffin; the paraffin sets more quickly than the sodium silicate solution. After 15 days of incubation most of the water-glassed stoppers were found to be quite firmly stuck to the tubes, a trouble not experienced with paraffined stoppers. The sodium coating is less flexible than one of paraffin, and it has a tendency to develop sharp projections on the corks, a drawback absent in the case of paraffin.

Corks coated with either sodium silicate or paraffin cannot be autoclaved without ruining the covering. It is possible, however, that water-glassed corks might be successfully sterilized in an oven. No significant difference in growth was observed between the fungus in tubes stoppered with water-glassed corks and that in tubes stoppered with paraffined corks.

Even with the use of the paraffined corks, there was some loss of moisture from the cultures, although appreciably less than from the cotton-plugged cultures. During an incubation period of about 6 months the average daily loss from the cork-stoppered tubes varied from 1.3 mgm. to 10.9 mgm. for the tubes containing the inoculated blocks and from 1.6 mgm. to 10.3 mgm.

for the controls. Corresponding figures for the cotton-plugged tubes are 16.8 to 48.6 mgm. and 15.5 to 42.9 mgm.

The tubes that were plugged with cotton contained agar gel about 4 or 5 cm. deep, whereas most of those with the cork stoppers had considerably less agar or practically none at all. It might be thought that perhaps the greater losses could be attributed to the fact that there was more moisture in the tubes. However, two tubes of a set with deep agar were stoppered with corks instead of cotton (with rubber covering) with the following results:

	Average daily losses	180-day incubation
	Inoculated	Control
Cotton-and-rubber-stoppered tubes (average of 4)	16.8 mgm.	15.5 mgm.
Cork-stoppered tubes	1.4	2.6

From these figures it is evident that the lesser losses from the cork-stoppered tubes are at least not entirely due to their smaller water content.

TABLE 1.—*Average daily loss in weight from cultures during an incubation period of about 6 months*

Original average moisture content of blocks, per cent (based on oven-dry weight)	Number of blocks averaged	Amount of loss, mgm.	
		Inoculated blocks	Control blocks
21	8	1.3	.....
24	2	.....	1.6
33	2	.....	2.7
35	8	2.8	.....
35	2	.....	3.5
38	2	.....	3.6
40	2	.....	3.4
46	8	3.7	.....
51	8	3.7	.....
53	2	.....	3.1
60	8	5.6	.....
65	2	.....	5.7
78	8	4.9	.....
80	8	4.8	.....
108	5	5.1	.....
113	5	5.5	.....
113	5	.....	6.1
120	5	.....	5.4
141	8	6.8	.....
143	2	.....	10.3

That there is, however, an approximate relation between the water content of the tube and the losses in weight is indicated by the figures of Table 1 obtained from a number of series of cultures prepared in a very uniform way, with only a small amount of agar in the tube, a piece about 0.5 cm. square placed upon the test block.

If figures for individual cultures be used, the irregularities are naturally found to be somewhat greater than in the averages just presented. For example, the loss from one inoculated culture, in which the original moisture content of the block was 107 per cent, was 10.1 mgm. per day, while, in another, with original moisture content of 111 per cent, the corresponding loss was only 2.9 mgm. For the control blocks of the series under consideration the figures are given in Table 2.

TABLE 2.—*Average daily loss in weight from individual control cultures during an incubation period of about 6 months*

Original moisture content of blocks, per cent	Amount of loss, mgm.	Original moisture content of blocks, per cent	Amount of loss, mgm.
24	1.6	58	5.4
24	1.6	60	1.4
28	2.3	72	5.9
34	3.4	105	7.9
35	3.7	114	8.7
36	3.1	116	8.5
36	3.8	118	1.7
38	3.1	124	4.2
40	3.3	137	4.8
43	3.6	171	12.0
45	4.8	.....	.....

It will be noted that, in spite of certain unexplained irregularities, the general trend of the figures indicates a rather direct relation between the amount of weight lost during incubation and the water in the preparation available for evaporation.

A comparison was made of the amount of weight lost by the tubes as a whole and by the blocks of the control preparations. Considering, first, the tubes containing a considerable amount of agar gel (about 20 ml.), it was found that the loss from the blocks was appreciably less than from the preparation as a whole. Evidently, there was a considerable loss of moisture from the agar gel. In a few instances the blocks gained in wet weight during the test period. This is undoubtedly due to an absorption from the agar, faulty technique allowing the blocks to come into contact with

the gel (*cf.* Hubert[7]). These tubes were stoppered with cotton and balloons.

In a number of series, the agar in the tube was considerably less than in those just discussed, varying from 0.5 ml. to 2 ml., mostly 1 ml. These tubes were stoppered with paraffined corks (or in one series with water-glassed corks). In some of these series the water loss from the blocks was greater than the loss from the preparation as a whole. This can readily be explained by assuming that the agar gel absorbed moisture that had vaporized from the block.

There are now to be considered the series in which almost no agar was used, the tubes being stoppered with paraffined corks. As in the case of the tubes containing large quantities of agar, there was, for the most part, a somewhat greater loss from the preparation as a whole than from the blocks, the difference averaging about 0.4 mgm. a day. Apparently there was some loss of weight from the stopper. It is possible that bits of solid paraffin, to the amount of 72 mgm., were lost from the preparation during the 6-month period, though it seems very unlikely. It is just as unlikely that such a loss is attributable to vaporization of moisture from the interior of the cork stopper at the temperature used or to the volatilization of constituents of the thin coating of paraffin used.

In these preparations containing a minimum of agar there seems to be a tendency, when the original moisture content of the block is more than about 117 per cent, for the block to lose more weight than the preparation as a whole. Since it is very unlikely that any appreciable amount of other materials than water is lost from the block during a 6-month period, this loss from the blocks must result in an increased humidity of the air within the tube.

In some tubes, containing 2 ml. of agar, or only a small amount, it was observed that dew had formed on the walls, presumably the result of condensation, when the tubes were taken out of the relatively warm culture chamber for inspection. Sometimes there was even free water in the bottom of the tube, 1–6 mm. deep. In a few cases free water was noted on top of 2 ml. of agar, below the mycelial mat when present. Such circulation of the water within the tube undoubtedly results in a slightly irregular distribution of moisture within the block, with a tendency for the outside to be a trifle moister than the inside. These differences, however, would be very difficult to demonstrate.

It seems that if paraffined stoppers are to be used in such work, special precautions must be taken to keep them firmly fixed in the mouth of the culture tube. It was noted time and time again, during routine monthly

[7] *Loc. cit.*, p. 34.

examination of the preparations, that some of the stoppers had become loose, rising above their former seating.

In a few cases, an appreciable gain (6–11 per cent) was noted in the oven-dry weight of the test blocks. Presumably, this is due to the absorption of solid substances from the agar gel by blocks in contact with it (*cf.* Hubert).[8]

Oxygen supply. The use of a relatively tight stopper introduces the problem of exhaustion of the oxygen in the culture tube with the subsequent slowing down of the activities of the fungus. One method suggested, by Hubert,[9] of overcoming this difficulty is to replenish the oxygen supply at intervals during the incubation period. Perhaps the simplest method of effecting this would be to open the tubes under aseptic conditions and decant the accumulated carbon dioxid, allowing a new supply of air to flow into the tube.

In view of the large capacity of the tubes used (78 ml. with stoppers in place) and the relatively small size of the test blocks (about 4 cm.[10]) it is possible that during a 6-month incubation period the oxygen exhaustion would not prove serious. This could be easily tested by preparing sets of similar cultures and decanting the carbon dioxid from some of the tubes at intervals, leaving some cultures till the end of the incubation period without such treatment; but this was not done in the studies under discussion.

When carbon dioxid is formed by the respiration of the fungus in a test-tube, it will, as is well known, settle to the bottom, and diffusion of the oxygen into this lower layer will be exceedingly slow. It is conceivable, therefore, that fungal growth might be inhibited at the bottom of a test-tube while there was still considerable oxygen in the upper part of the tube. It seems certain that such a result could be postponed by elevating the test block above the bottom of the tube, as on a short section of thin glass tubing. One set of tests with this method, however, yielded inconclusive results.

It might be thought that an absorbent of carbon dioxid, such as soda lime or potassium hydroxid, could be used in the bottom of a culture tube to take up the carbon dioxid as formed, allowing the remaining oxygen to reach the growing fungus. Unfortunately, these substances absorb water as well as carbon dioxid, and test blocks placed in tubes containing them lose moisture to a very pronounced extent.

The following specific figures will make this clearer. In 5 sets of 5 non-inoculated blocks, each, varying in average moisture content per block from 110 per cent to 174 per cent, and placed in tubes containing soda lime,

[8] *Loc. cit.*, p. 34.

[9] *Loc. cit.*, p. 38.

[10] Humphrey, *loc. cit.*

the average moisture content at the end of 6 months ranged from 11 per cent to 26 per cent. In these tests the average daily loss in weight from the blocks was considerably in excess of that from the tubes, ranging from 2 to 13 mgm. more. However, from each of the 5-tube sets there was a net loss amounting to an average per tube of 3 to 9 mgm. a day. While the available figures are not numerous enough to show the fact definitely, there is an indication that this net loss is very close to that from the tubes with almost no agar and, presumably, is attributable to the same causes, whatever they may be. At any rate, it seems evident that under the conditions of these tests, the soda lime does not absorb an appreciable amount of carbon dioxid or water from the air surrounding the tubes.

In these tests the soda lime was placed in short lengths of glass tubing, about 8 cm. long, plugged at each end with cotton (or of glass wool or asbestos fibers) and then, having been placed in the test-tubes, sterilized and dried in an oven. After as short an incubation period as 30 days it was noted that the soda lime was deliquescing and saturating the lower cotton plug. At the end of 2 months there was free liquid in the bottom of some of these culture tubes to a depth of 0.5 to 1.0 cm. These effects were less marked in the case of glass-wool plugs, and still less so in the case of asbestos. There appeared to be less dew on the test-tube walls in the presence of soda lime than in comparable preparations without the soda lime.

Inoculation methods. Several different methods of inoculating the test blocks were tested. In the earliest work, the fungus was established, during a period of a week or 2, on about 20 ml. of 1.5 per cent nutrient agar gel in the bottom of a test-tube. Small sterile glass plates, about 1.3 cm. square and a couple of millimeters thick, were then placed upon the gel and the test blocks introduced on top of these. This method resulted in good fungal growth on the blocks. In the case of the control blocks, however, there was a tendency for the plates to sink down into the gel, allowing the blocks to absorb moisture from the agar. It may be that the inoculated blocks did not so absorb moisture because of the support offered by the fungal mat on the surface of the agar. On the other hand, it seems possible that water is brought up through the fungal hyphae themselves, when they are in contact in this way with a moist mass of agar gel.

Two glass plates as supports proved no more satisfactory than one in preventing increase of moisture content. When the glass plates were supported on a glass tube driven down into the agar gel, however, the increase in moisture content did not occur. The use of more concentrated agar (3 per cent) with glass plates on top also eliminated moisture absorption by the blocks.

Still the question remained as to whether a smaller quantity of agar gel might not be preferable, and several sets of cultures were prepared with 2

ml., 1 ml., or 0.5 ml. of agar gel. A test of a couple of air-dry blocks placed directly upon 1 ml. of 1.5 per cent agar indicated that there would be inappreciable absorption of moisture by blocks under such conditions. As it turned out, there was a consistent loss of moisture, during the 6-month incubation period, from the blocks in the tubes with small amounts of agar.

Another method of inoculating involved the use of a small bit of mycelium, about 0.5 cm. square and perhaps half a mm. thick, placed directly upon the block in the bottom of the test-tube, or sometimes upon the wall of the test-tube, adjacent to the block. The conrols for such test blocks were provided with bits of nutrient agar of approximately the same dimensions.

Infection of the test blocks resulted from all methods of inoculation, although not in all instances. In a few cases abortive sporophores were produced within 6 months, both when 1 or 2 ml. of agar gel were placed in the tube and when a small amount of inoculum was used.

When the inoculum is placed upon the tube wall rather than upon the block, it seems desirable to leave the preparation lying on its side for about 2 days, with the block in contact with the infected agar gel.

Sterilization. The agar solution was sterilized in the autoclave, at relatively low pressures, in accordance with customary laboratory technique, cotton plugs being used for the test-tubes. Glass plates, glass tubes, and soda lime were usually baked for a number of days in a 100° C. oven. Occasionally, the plates were dipped in ethyl alcohol, which was then burned off. When blocks were to be placed in empty test tubes, these, too, with cotton plug, were oven-sterilized.

Sterilization of the blocks was considered effected during the process of adding water by autoclaving at 98.5° C. That this treatment probably had the desired result is indicated by the facts that no wood-destroying fungi were noted on the control blocks and that significant losses in oven-dry weight were not observed for the control blocks. It is not certain that the method would be applicable to the study of resinous heartwoods.

The most troublesome problem of sterilization concerned the stoppers. As Hubert[11] has mentioned, mold is apt to develop in capped cotton plugs. If the upper walls of the tube bear the slightest trace of nutrient agar, these molds are very likely to creep down the walls and cause contamination of the agar at the bottom and of the test block.

It was thought that the use of paraffined corks might obviate this difficulty. The paraffin was not hot enough to sterilize the corks, but they were habitually dipped in ethyl alcohol and allowed to dry before substituting them for the cotton plugs used while sterilizing the culture tubes. In spite of this treatment, molds of unidentified genera, but probably species of Penicillium, Rhizopus, and Aspergillus, developed upon the por-

[11] *Loc. cit.*, p. 38.

tion of the stopper within the tube in a great many cases and spread to the agar and to the blocks even in preparations where there was almost no agar in the tube. One set of tubes stoppered with freshly paraffined corks not dipped in alcohol seemed no worse contaminated than when the alcohol bath was used.

It is possible that contamination of the stoppers took place while the alcohol was drying from them. In view of results from exposures of nutrient agar under somewhat similar circumstances, however, this explanation seems somewhat untenable. Another possible explanation is that the mold spores were on and perhaps some distance inside the stoppers, that they were protected from the alcohol by the paraffin coating, and that the mycelium eventually grew through the paraffin coating. It is desirable to try oven-sterilizing of the corks just prior to the paraffin treatment.

MISCELLANEOUS DETAILS OF TECHNIQUE

In the manipulations involved in these studies, a number of practices and conventions were adopted and are here recorded as suggestions for future workers on similar problems.

When cotton plugs were replaced by paraffined corks, any adhering bits of cotton were habitually removed from the throat of the tube by means of sterile forceps.

In making the preparations, the blocks were weighed in the air-dry condition and a computed amount of water added on the basis of the summed weights. The air-dry moisture content of the blocks was approximated by ascertaining the moisture content of similar blocks from time to time. The culture tubes were weighed with agar, glass plates, etc., and cotton or cork stoppers just before adding the blocks and again immediately afterwards. The difference in weight gave the wet weight of the block. The moisture content was then computed on the basis of this weight, the air-dry weight, and the air-dry moisture content. When rubber caps were used, the tubes were weighed a third time after the addition of these.

Gross-weight weighings of the preparations were made at intervals during the incubation period, usually not oftener than once a month. At the completion of incubation the fungus was removed from the outside of the test blocks and the block weight ascertained.

SIGNIFICANCE OF MOISTURE CONTENT

On the basis of the study as conducted to date, no attempt at a statistical treatment of the results from the standpoint of favorable moisture content would be profitable because of the failure to develop a technique for maintaining the moisture content reasonably uniform. It is impossible, at the present stage of this study, to make any generalized statement about optimal or minimal moisture content. Inasmuch, however, as active growth

TABLE 3.—*Loss in oven-dry weight of inoculated blocks under various moisture conditions during a 6-month incubation period, with paraffined-cork stoppers, except as otherwise noted*

Loss of weight, per cent	Moisture content, per cent		Method
	Original	Final	
0	39	8	Small inoculum
0	39	6	" "
0	45	39	Deep 3% agar gel, glass plate
0	48	34	2 ml. 1.5% " " " "
0	51	36	2 ml. 1.5% " " " "
0	72	9	Small inoculum
0	80	50	1 ml. 1.5% agar gel, glass plates
0	97	74	1 ml. 1.5% " "
0	110	97	0.5 ml. 1.5% " " glass plate on tube walls
0	117	89	1 ml. 1.5% " "
5	66	7	Deep 3% " " glass plate; cotton-and-rubber stopper
5	69	6	1 ml. 1.5% agar gel
5	74	6	1 ml. 1.5% " " glass plate
5	79	69	1 ml. 1.5% " " " "
5	95	26	0.5 ml. 1.5% " " " " on tube walls
5	102	37	0.5 ml. 1.5% " " " " " " "
5	146	33	Deep 3% " " " " cotton-and-rubber stopper
5	153	19	Small inoculum; soda lime (cotton)
5	190	8	" " " " (glass wool)
10	70	7	Deep 3% agar gel, glass plates; cotton-and-rubber stopper
10	81	6	1 ml. 1.5% agar gel
10	108	34	Deep 1.5% " " glass plates; cotton-and-rubber stopper
10	108	31	Deep 1.5% agar gel, " " " " rubber stopper
10	114	32	Deep 1.5% agar gel, " " " " rubber stopper
10	117	7	2 ml. 1.5% agar gel, " "
10	126	39	Small inoculum
10	136	37	Deep 3% agar gel, glass plate; cotton-and-rubber stopper
10	154	97	2 ml. 1.5% agar gel, glass plates
10	192	146	2 ml. 1.5% " " " "
14	49	36	1 ml. 1.5% " " " "
14	138	76	2 ml. 1.5% " " " "
14	148	69	2 ml. 1.5% " " " "
15	54	43	2 ml. 1.5% " " " "
15	135	162	Deep 1.5% " " " " cotton-and-rubber stopper
15	137	233	Deep 1.5% agar gel, " " " " rubber stopper
16	108	66	1 ml. 1.5% agar gel, " "
16	118	49	Small inoculum
16	130	167	Deep 1.5% agar gel, glass plates; cotton-and-rubber-stopper
19	99	32	1 ml. 1.5% agar gel
21	128	286	Deep 1.5% agar gel, " " " " rubber-stopper
30	131	161	Deep 1.5% agar gel, " " " " rubber-stopper
31	131	227	Deep 1.5% agar gel, " " " " rubber-stopper

of the test fungus was obtained under a number of conditions, it will be of interest to present a few of the observations. These are given in tables 3 and 4.

TABLE 4.—*Showing conditions under which abortive sporophores were produced during a 6-month incubation period, with paraffined-cork stoppers*

Moisture content, per cent		Loss of weight, per cent	Method
Original	Final		
43	14	4	Small inoculum
54	35	7	" "
64	39	7	" "
81	6	10	1 ml. agar gel
83	14	11	1 ml. " "
85	7	12	1 ml. " " glass plate
86	31	17	1 ml. " "
89	41	25	1 ml. " "
92	37	13	1 ml. " "
99	46	23	1 ml. " "
99	32	19	1 ml. " "
117	7	7	2 ml. " " " "
118	49	16	Small inoculum

SUMMARY

This paper describes results of a study of suitable methods to use in investigating the relation between moisture content of wood and the activity of decay-producing fungi. In this study *Fomes roseus* was grown on sapwood of *Pinus ponderosa* at a temperature of about 27° C. for approximately 6 months.

By adding water to test blocks in a stender dish, autoclaving them the following day at atmospheric pressure, and allowing them to stand another day, uniform moisture distribution was obtained. No method, however, was discovered whereby the desired amount of moisture could be added to a test block, except approximately.

The use of small bits of mycelium gave indications of being a satisfactory method of inoculating test blocks.

Tests of methods of stoppering culture tubes have progressed to a fairly satisfactory point. The use of paraffined corks promises good results, but more study is still needed on this problem.

The autoclaving incident to the process of adding moisture to the test blocks proved a satisfactory method of sterilization for the test materials used and under the conditions of experimentation adopted.

These studies failed to yield specific information on the relation of moisture content to activity of wood-destroying fungi.

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VARIETAL SUSCEPTIBILITY OF BEANS TO AN AMERICAN AND A EUROPEAN STRAIN OF PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA, AND A COMPARISON OF THE STRAINS IN CULTURE

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Although the disease of beans caused by *Phytomonas medicaginis* var. *phaseolicola* Burkh. has been known for only 5 years, it is already recognized as the most serious bacterial trouble of this crop. Burkholder (3, 5), Hedges,[2] Higgins (6), and Zaumeyer[3] have described its severity of attack in various sections of the United States. Furthermore, the disease is not limited entirely to America but, in Europe, it has been reported from Germany by Stapp and Kotte (9), from Holland by Wieringa (10), and from Switzerland by Burkholder (5).

While the severity of the disease has been emphasized by the above-mentioned American investigators, little has been written concerning the various degrees of susceptibility that our American commercial varieties of beans exhibit toward the trouble. It has been observed in New York that a definite amount of resistance could be found in certain varieties, and Higgins (6) has reported this to be true in his work with 10 varieties of beans in Georgia. More extensive varietal-susceptibility tests have been conducted in Europe by Wieringa (10) and more recently by Kotte (7). The varieties of beans used by these two European investigators, however, are not grown in America, so that their published results are not very helpful to workers on this side of the Atlantic.

For some time it has been felt that further knowledge concerning the varietal susceptibility of American commercial beans of both the dry-shell and snap varieties was desirable. Such information could be used not only in extension work among the growers but as a basis for breeding work to secure desirable resistant varieties. The present investigation is an outcome of this need. The work reported in this paper, however, is not limited entirely to this phase of the disease problem but is coupled with the

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[2] Hedges, Florence. Bacterial diseases of beans in some western commercial seed-growing and canning areas and southern trucking sections in 1927 and 1928. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. **12**: 121–122. 1928.

[3] Zaumeyer, W. J. Bean diseases in western United States in 1929. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. **14**: 38–43. 1930.

problem of whether or not the American and European strains of *Phytomonas medicaginis* var. *phaseolicola* are identical.

METHODS OF INOCULATION

Before proceeding directly to the varietal-susceptibility tests, a discussion is pertinent here as to the most satisfactory method of infecting the bean plants with the pathogen under consideration. Such a method should be one in which the severest and most uniform infection could be secured. The senior writer had experienced some difficulty during the previous season in obtaining uniform infection on varieties in the field, so, in this case, greenhouse trials were decided upon. Maturing seedlings or young plants approximately 15 cm. in height were used and three methods of inoculation were tried in an extensive series of experiments. It should be stated that these comparative infection tests were run at the same time that two of the main varietal susceptibility experiments were conducted. They are treated separately here only as a matter of convenience. Thirty-four varieties of beans were used in these tests and are the first 34 listed in table 1. They were run in duplicate, since two different strains of *Phytomonas medicaginis* var. *phaseolicola* were used, and, furthermore, the entire experiment was repeated. Thus, a sufficient amount of data was obtained from which to draw fairly adequate conclusions on the methods used.

Each of the 34 varieties was divided into 3 groups of approximately 4 plants each. One group of plants was inoculated by spraying with a water suspension of the bacteria, while the plants in the second group were injured at the cotyledon node and the inoculum inserted into the wound. The third group was injured and inoculated at the first leaf node. In the methods where the plants were injured the procedure as described by Burkholder (5) was followed. In all cases the pathogen was grown on agar slants at 24° to 27° C., and 2- to 3-day-old cultures were used. When the plants were inoculated by injuring them the bacteria were removed from the agar on the tip of a sharp pointed scalpel and the plants were stabbed with this contaminated instrument. In these trials no special care of the plants was taken; that is, they were allowed to remain on the greenhouse benches before and after inoculation. When the plants were inoculated by spraying with a water suspension of the bacteria from agar slants they were first placed in a moist inoculating chamber for 24 hours, as recommended by Smith and others, and, after spraying, were again placed in the inoculating chamber. One group of plants was allowed to remain under these moist chambers for 24 hours after inoculation and a second group for 48 hours.

The first of these comparative experiments was conducted in the greenhouse in July, 1930, and the second set in August, same year. Under the

TABLE 1.—*Showing varietal susceptibility of American commercial varieties of beans to two American strains and one European strain of Phytomonas medicaginis var. phascolicola*

Varieties	First trial		Second trial		Third trial	
	1st American strain	European strain	1st American strain	European strain	2nd American strain	European strain
Black Valentine	Moderate	Slight	Very Slight	Slight	Very Slight	Very Slight
Bountiful	Severe	Very Severe	Moderate	Very Severe	" Severe	Moderate
Burpee's Kidney	"		Slight	Moderate	Slight	Very Slight
Burpee's Stringless	Moderate	Severe	Very Slight	Severe	Severe	Moderate
Currie	"	Moderate	None	Moderate	Slight	Very Slight
Davis Wax	"	"	Slight	"	None	None
Dwarf White Kidney	Severe	"	Moderate	Slight	Slight	"
French Horticultural	Very Slight	"	Very Slight	Moderate	"	Slight
Giant Stringless	Severe	Severe	Moderate	Severe	Severe	"
Golden Eyed Wax	Moderate	Moderate	Very Slight	Moderate	Moderate	"
Hodson Wax	"	Very Severe	Slight	"	Very Severe	"
Improved Golden Wax	Severe	Moderate	"	"	" "	Moderate
King of the Earlies	Very Slight	Slight	Very Slight	"	Slight	Slight
Longfellow	Severe	Very Severe	Slight	Very Severe	Very Severe	"
Low Champion	Very Severe	" "	Severe	" "	" "	Severe
Pencil Pod Black Wax	Moderate	Moderate	Very Slight	Slight	Slight	None
Pod Spot Proof Wax	Very Slight	"	" "	Moderate	"	Very Slight
Refugee 1000–1	Moderate	Slight	" "	None	None	None
Refugee Wax	Severe	Moderate	None	Slight	Very Slight	"
Red Kidney	"	Very Severe	Very Severe	Very Severe	" Severe	Moderate
Red Valentine	Moderate	Severe	Slight	Slight	" Slight	None
Roger's Stringless Refugee	"	Moderate	None	Very Slight	None	"
Round Pod Kidney Wax	Slight	Slight	Very Slight	Moderate	Very Slight	Very Slight
Ruby Horticultural	Moderate	Severe	Moderate	Severe	Severe	Moderate
Scotia	Very Slight	None	None	None	None	None
Simmons Stringless Round Pod	Very Severe	Very Severe	Severe	Very Severe	Very Severe	Slight
Sure Crop Wax	Slight	Moderate	Very Slight	Slight	" Slight	Very Slight
Tennessee Green Pod	Very Slight	Slight	None	None	None	None
Unrivalled Wax	Severe	Severe	Slight	Severe	Severe	Slight

TABLE 1.—(*Continued*)

Varieties	First trial		Second trial		Third trial	
	1st American strain	European strain	1st American strain	European strain	2nd American strain	European strain
Wardwell's Kidney Wax	Severe	Very Severe	Moderate	Very Severe	Very Severe	Moderate
Webber Wax	"	Severe	"	" "	" "	Slight
White Marrow	Moderate	"	Very Slight	Severe	" "	Moderate
White Kidney	Very Severe	Very Severe	Moderate	Very Severe	Severe	Slight
Yellow Eye	Moderate	Severe	Slight	Slight	Very Slight	Very Slight
Blue Pod Medium					None	None
Challenge Black Wax					Slight	Very Slight
Full Measure					Severe	Slight
Kentucky Wonder					None	None
Lazy Wife					Severe	Severe
Perry Marrow					Moderate	Very Slight
Otenashi					None	None
Robust Pea					"	"
Sure Crop Wax					"	"
Well's Red Kidney					Very Severe	Moderate

conditions of the experiments the results demonstrated that stabbing and inoculating the plants at the first leaf node gave a more consistent and a greater degree of infection than the other methods employed. This was evident not only on those varieties showing an extreme susceptibility but also on those that displayed a certain amount of resistance. It was to be expected that the disease would progress more rapidly in the plant and become more severe when the organism was introduced directly into the xylem. It is in this tissue that the invasive ability of the bacteria is greatest. When the bacteria were sprayed on the leaves their entrance through the stomates and establishment in the tissue were somewhat slower. Also, a further disadvantage of the spray method, one of greater importance for a varietal susceptibility test, was the fact that infection was not uniform even among individuals of the same variety. The causes of these irregularities probably are many, but an uneven distribution of inoculum, no doubt, is a primary factor.

Infection took place more quickly and the symptoms of the disease became more severe when the plants were inoculated at the first leaf node than at the cotyledon node. This difference in degree of infection seems due merely to the position of the inoculation court. The first leaf node was more centrally located.

Some attempt was made to conduct comparative infection experiments on the pods, but the data collected were few and are not given here. The pods of the different varieties were seldom produced at the same time; consequently, the results were not comparable.

VARIETAL SUSCEPTIBILITY TESTS

In conducting experiments to determine the varietal susceptibility of the more common commercial varieties of beans 3 different tests were made. The first experiment was carried on in July; the second, in August; and the third extended from the middle of October into November. The temperature during the summer months was variable and at times was as high as 35° C. at noon. During October and November the greenhouse in which the experiments were conducted ranged from 25° to 29° C. This fairly constant temperature held in the last experiment was maintained through the use of automatic devices installed in the greenhouse.

Thirty-four varieties of beans were used in the first 2 experiments, but 9 additional varieties and 1 duplication were added in the final experiment. In the first test the beans were grown in flats (45 x 32 cm.) in 3 rows of 8 each, but in the following experiments 6-inch pots were used. The last method was more satisfactory, since the plants had more soil and made a better growth. The number of plants of each variety inoculated varied

somewhat due to the fact that at times there was a poor germination of seed. Seldom, however, were there less than 8 plants in a variety, and in the majority of cases there were 10 or 12. This number does not include the plants inoculated by spraying and those inoculated by stabbing at the cotyledon node. Only those inoculated by injury at the first leaf node were considered in the varietal-susceptibility experiments.

Since the trials were conducted 3 times, approximately 25 individuals were used in each of the 34 varieties. The number of each of the 10 varieties added in the last experiment was 8 to 12.

In the first 2 sets of experiments 2 different strains of *Phytomonas medicaginis* var. *phaseolicola* were used, one of American and the other of European origin. The latter strain was obtained through the courtesy of K. F. Wieringa, who had isolated it from material collected in Holland. The American strain was isolated from a Red Kidney bean pod collected in New York State in 1924, but it had been inoculated repeatedly into bean plants and reisolated since then. Both strains appeared to be virulent. In the third trial the Holland strain was still used but the American strain referred to above was discarded for a second strain isolated from bean leaves collected in New York in September, 1930.

The plants were inoculated usually about 10 to 14 days after planting or when they were approximately 15 cm. in height. Three weeks after inoculation the plants were carefully examined to determine the amount of infection and the data for all 3 sets of experiments are recorded in table 1.

This time of examination was not chosen arbitrarily but through careful observation. The progress of the disease on the young plants was watched from the time of its appearance until a month or so had elapsed after inoculation. At the end of three weeks degrees of infection were, as a rule, very distinct but, after this time, there was a mingling of certain adjacent degrees.

In determining the degree of susceptibility of each variety a method similar to that used by Barrus (2) and by Rands and Brotherton (8) was employed. Six classes or degrees of infection were adopted and are described below. A description of the symptoms of the disease is not given here, since they have been described in full, with illustrations, by the senior writer (5) and a repetition appears unnecessary. Following are the degrees of infection.

None. There is no evidence of any symptom of disease or only numerous small local lesions appear.

Very slight. Small lesions of sufficient number do at times cause the death of a single leaf.

Slight. The first leaves are destroyed but the plant appears to outgrow the infection and at the end of 3 weeks there is little evidence of the disease.

Moderate. Systemic infection is evident. A moderate stunting of the plant and at times chlorosis of the leaves take place. The lower leaves, as a rule, are lost.

Severe. The symptoms due to a systemic infection are very evident. Plants are stunted approximately 33⅓ per cent. Leaves are dwarfed, chlorotic, and wrinkled. At the end of 3 weeks plants are alive, although there is no promise of even a moderate crop of seeds.

Very severe. Plants are dead 3 weeks after inoculation.

In examining table 1 it may be seen that there is some variation in the degree of susceptibility of a variety occurring in the different trials and between the different strains, even in the same trial. These variations are never consistent, however, and may be explained here on the basis of environmental factors and differences in virulence of the organisms. Certain of the trials and varieties should be commented upon, however. In the first trial the infection, on the whole, appears to be slightly more severe than in trials two and three. This apparent difference is due possibly to the fact that these plants were growing in flats with little soil and in an environment where the tendency was for the soil to become dry and compact. Thus, the plants were weakened and infection appeared greater than it probably really was. The American strain used in the third trial was recently isolated and appeared at times somewhat more virulent than the strain used in trial two. On the whole, however, no consistent difference can be seen between the reactions of the varieties of beans to the American strain and to the European strain of *Phytomonas medicaginis* var. *phaseolicola*. Those varieties that show a great susceptibility to one strain also exhibit this characteristic to the other strains.

Considering the table as a whole, one can readily point out the varieties of bean that are very susceptible to the disease and those that show a great amount of resistance. Since there were three trials and several strains of the organism used, the data should yield results of a fair degree of accuracy.

The varieties of beans that are very susceptible to the disease are as follows: Bountiful, Giant Stringless, Improved Golden Wax, Longfellow, Low Champion, Red Kidney, Ruby Horticultural, Simmons Stringless, Round Pod, Unrivalled Wax, Wardwell's Kidney Wax, Webber Wax, White Kidney, Lazy Wife, and the Well's Red Kidney.

Those varieties that show a great amount of resistance to or tolerance for the disease are: Black Valentine, French Horticultural, King of the Earlies, Pencil Pod Black Wax, Pod Spot Proof Wax, Refugee 1000–1, Refugee Wax, Rogers Stringless Refugee, Round Pod Kidney Wax, Scotia,

Sure Crop Wax, Tennessee Green Pod, Blue Pod Medium, Challenge Black Wax, Kentucky Wonder, Otenashi, and Robust Pea. The 12 remaining varieties probably should be referred to as moderately susceptible.

The majority of those varieties listed above as very susceptible to *Phytomonas medicaginis* var. *phaseolicola* have been tested also for their susceptibility to *Phyt. phaseoli* by Burkholder (4) and by Rands and Brotherton (8). In these two latter reports this group of varieties also appears to be very susceptible to *Phyt. phaseoli.* One would think there was a correlation between susceptibility to the two diseases. On the other hand, however, the varieties of beans found by the writers to be but slightly affected by *Phyt. medicaginis* var. *phaseolicola* may or may not be very susceptible to *Phyt. phaseoli*, and the correlation that appeared in the first group is not found in the second group. It is true, nevertheless, that such common bean varieties as the Refugee 1000–1, Roger's Stringless Refugee, Robust Pea Bean, Scotia, and French Horticultural show resistanance to both organisms, but this rule does not follow with certain of the other varieties. The Black Valentine may be cited as such an example. Both Higgins (6) and the writers list this variety as only slightly affected by *Phyt. medicaginis* var. *phaseolicola,* but Burkholder (4) and Rands and Brotherton (8) state that it is severely affected by *Phyt. phaseoli.* This condition also is similar to that in certain other varieties.

Wieringa, in his work on varietal susceptibility of beans to the disease, found a correlation between susceptibility and purple blossoms. Kotte (7) did not observe this correlation, and it was not found by the writers in this present investigation.

COMPARISON OF THE VARIOUS STRAINS OF THE PATHOGEN IN CULTURE

Although the varietal susceptibility experiments reported above indicated that the European strain of *Phytomonas medicaginis* var. *phaseolicola* is identical with the American strains, at least on a pathogenicity basis, it was considered worth while to make a comparative test of the organisms in pure culture. In conducting these tests with the 3 strains of the pathogen used above, it soon was apparent that they coincided in appearance and in reaction in the various media. The morphology of each was similar, as also were their growth characteristics in such common media as beef-extract bouillon and agar, milk, and Uschinsky's solution. There was a slow liquefaction of gelatin, nitrates were not reduced, indol was not formed, and there was only slight, if any, hydrogen sulphide produced. All 3 strains were facultative anaerobes, as shown in dextrose broth in fermentation and in shake agar cultures.

Extensive carbohydrate-fermentation studies were conducted with the hope of finding differences between the strains, but the results were negative. Since the majority of substances used in these experiments, however, have not been reported upon before for this organism, they are given here in some detail. In the main, the basic medium used was the modification of the solution used by Ayers, Rupp, and Johnson and recommended in the *Manual of Methods for Pure Culture Study of Bacteria* issued by the Society of American Bacteriologists. The bacteria grew well in this simple solution, showing that they could use inorganic nitrogen; and, since the carbohydrate to be tested was the only source of energy for the organism, the determinations of the fermentation of the substance were not complicated. Brom-Creosol-purple was used as an indicator, and all carbohydrates were sterilized by autoclaving with the exception of lactose, maltose, raffinose, and sucrose. These 4 sugars were filtered. The recommendations of Ayres, Rupp, and Johnson (1) were followed in preparing the organic-acid media. In the fermentation of starch the starch-agar-iodine method was used.

All 3 strains fermented the following carbohydrates but without the production of gas; dextrose, levulose, galactose, mannose, arabinose, xylose, sucrose, glycerol, citric acid, and malic acid. The following were not fermented; rhamnose, lactose, maltose, raffinose, mannitol, salicin, starch, acetic acid, benzoic acid, lactic acid, and tartaric acid.

SUMMARY

Experiments were conducted to determine the varietal susceptibility of a number of American commercial varieties of beans to the disease caused by *Phytomonas medicaginis* var. *phaseolicola*. Two American strains and one European strain of the pathogen were used. There were no differences in the susceptibility of varieties to the different strains. Certain varieties, however, proved to be considerably resistant to the disease, while others displayed various degrees of susceptibility.

The European strain of the pathogen was compared in pure culture with the American strains. No differences were noted.

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LITERATURE CITED

1. AYERS, S. H., P. RUPP, and W. T. JOHNSON. A study of the alkali-forming bacteria found in milk. U. S. Dept. Agric. Bul. 782. 1919.
2. BARRUS, M. F. Varietal suspectibility of beans to strains of *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. & C. Phytopath. **8**: 589–614. 1918.

3. BURKHOLDER, W. H. A new bacterial disease of the bean. Phytopath. **16**: 915–927. 1926.
4. ————————. Varietal susceptibility among beans to the bacterial blight. Phytopath. **14**: 1–7. 1924.
5. ————————. The bacterial diseases of the bean: a comparative study. Cornell Agr. Exp. Sta. Memoir 127. 1930.
6. HIGGINS, B. B. "Halo Spot" of beans and kudzu. Ga. Exp. Sta. Bul. 161. 1930.
7. KOTTE, WALTER. Zur Kenntnis der "Fettfleckenkrankheit" der Bohne. Ztschr. Pflanzenkr. **41**: 12–19. 1931.
8. RANDS, R. D., and W. BROTHERTON (JR.) Bean varietal tests for diseases resistance Jour. Agr. Res. **31**: 101–154. 1925.
9. STAPP, C., and W. KOTTE. Die Fettfleckenkrankheiten der Bohne, eine für Deutchland neue durch Bakterien herforgerufene Pflanzenkrankheit. Nachrichtenbl. Deut. Pflanzenschutzd. **9**: 35–37. 1929.
10. WIERINGA, K. T. De vetvlekkenziekte, een voor Nederland nieuwe ziekte bij Bruine Boonen (*Phaseolus vulgaris*). Tijdschr. Plantenziekten **36**: 84–87. 1930.

IMMUNITY IN PLANTS

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While we may find it easy to describe all of a series of immunological facts, we may not find it so easy to agree on a definition of immunity. Immunity, as we understand it, is applied to that animal that does not contract an infectious disease to which it is susceptible even though it comes in contact with the microorganism or the agent of the disease. Thus, it is evident that we are considering only the pathogenic power of the agent rather than the faculties of the agent to enter into a symbiosis, either mutualistic or parasitic. In animals illustrations of this sort are frequent, not only in their open organs that communicate with the external world directly, as the intestines, or, indirectly, as the cystiphellea, but also in their closed organs, such as the circulatory system. In the case of healthy carriers the microorganisms, pathogenic and virulent in themselves, are harbored for long periods without evincing any apparent harm. Common examples applicable to open organs of the human carrier are: bacilli of tuberculosis and of diphtheria in the respiratory tract; vibrios of cholera; and typhoid bacilli in the intestinal tract. On the other hand, one finds the treponema of syphilis practically anywhere in man, and, in 1923, De Kock informed us from South Africa that horses can carry the virus of pernicious anemia in their blood as long as 7 years after the cure (10).

The above concepts are useful and practical in the field of plant immunology, as well as in medical bacteriology. In fact, one should make evident that some of the most characteristic examples of immunological reactions in plants are drawn from the chapter on symbiosis, not, however, from the parasitic but from the mutualistic. It is for this reason that the Italian school has introduced the term refractoriness, to indicate the absolute impossibility of any relationship of life in common and to mean by immunity the act of hindering the symbiont from becoming pathogenic.

Among the forms of immunity that we are accustomed to distinguish there are, primarily, general and local immunity. One of the chief objections to phytoimmunity is that in plants one cannot think of an immunization of the whole organism because of the great independence of their parts.

Before stating the facts that answer the objection, it will be opportune to note at this point that most recently we have learned more and more to acknowledge and to evaluate in just terms the importance of strictly local immunizations, even in animals. Citing a typical case, we have the work of Altana, who infected the cornea of a rabbit with smallpox vaccine (1). After the healing of the specific lesion, the affected part enjoyed an out-

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standing immunity not at all shared by the rest of the body, which continues in its own state of susceptibility. The establishing of a general immunity, on the other hand, does not protect the cornea from being susceptible to successive vaccinations.

Another important objection is based on the fact that the fluids of plant cells are generally acid, while those of animal cells are alkaline. It is claimed that this difference is so fundamental that we can not assume any analogy between the behavior of plant and animal cells. However, acidity and alkalinity are only relative terms. The pH of animal fluids varies over a wide range and is only a continuation of the range of pH covered by plant juices. Lately, the study of immunological facts of the rudimentary animals seems to have resulted in extremely interesting observations of the few who have ventured into this field, Cantacuzene and Metalnikoff (7, 14). We find statements of irrefutable value where imperceptible steps are established by endless gradations from the animal to the vegetable realms. More time should be spent on the interesting chapter on immunology in Protozoa and Metazoa up to the insects, but, regretfully, space permits but a mere mention of them. Note the single case of that Tunicata, *Ascidia mentula*, which is an animal whose blood has an acid reaction like plants.

As for the plants, we all know that the most exact knowledge of the degree of resistance of cultivated plants to the most serious diseases is of a very practical value, chiefly in relation to the variability of resistance of singular races. This makes possible a choice among them, according to the environment, and it can constitute, furthermore, one of the cardinal points in the perfecting of artificial selection. However, we know a great deal less about the nature and mechanism of congenital immunity in plants. Certainly theories have been formulated, but all of them sin in one-sidedness, either because they seem tentative in simple adaptation through analogy with animal immunology or because they are based upon isolated specific observations that do not yet lend themselves to generalizations. If we consider essentially the distribution of immunity in plants and the principal fact of the specialization of parasites, the factors of resistance in plants can be thus classified: (1) Mechanical defenses dependent upon the anatomical structure of tissues. (2) Actions of normal cellular and intercellular fluids before the parasitic attack. (3) Histogenic and humoral reactions of tissues under the action of the parasites.

From 1911 to 1919 Vavilov (19) thoroughly studied the question of the distribution of immunity and its connection with the specialization of the parasite. He concluded that the more limited the field of receptibility of a given fungus, the easier it is for this to be susceptible to the different reactions of some varieties; the more likely it is to find varieties in the same species that are immune or at least much less susceptible. *Sclerotinia tri-*

foliorum Eriks. attacks Medicago, Onobrychis, and some other genera of the Papilionaceae; it is very little specialized and consequently no varieties of immune clover are observed. But the rust known as *Melampsora lini* Boll. is incapable of affecting anything other than the individuals of the genus Linum. Accordingly, there really are varieties of flax perfectly resistant to this rust.

Without considering the subtle distinctions among nonspecialized parasites in the so-called physiologic forms, where many points are still uncertain, Stakman and his collaborators distinguish approximately 100 physiological forms of *Puccinia graminis* Pers. in this country (18). It is worth while, however, to call attention to the fact that the distribution of immunity in a single given species is particularly related to the genetic differentiations of the varieties.

Triticum monococcum L., a species of wheat, resists in all its varieties the brown, the yellow, and the black rusts—a fact that distinguishes it particularly from any other wheat. Genetically, *Triticum monococcum* occupies a place distinct among wheats; and it does not yield fertile hybrids with other species.

Passing in rapid review the mechanical factors of congenital immunity, we find it important to mention the specific action of plant juices. Furthermore, we also find it rather easy to understand the functioning of the hundreds of means of defense exploited by nature from the number of stomata on the leaves and the different movements of stomatal cells to the infinite forms of flowers and to suberifications of the cortical cells and to the thickness of the sap.

The natural defense is based on a few well-known elements and on many not so well known; and it still represents a magnificent field for investigations. The Americans have of late devoted a great deal of attention to it; their collected data have value of noteworthy significance.

The organic acids more or less diffuse in plants can inhibit parasitic growth; *Bacillus carotovorus* L. R. Jones infects the tomato quite easily but does not attack oranges or lemons. Nobécourt has proved and stated this fact *in vitro*, using sterile extracts, raw and boiled, of the above-mentioned fruits (15). The action is identical with that of a solution of citric acid of the same concentration, but, thus considered, the assertion seems too obvious, because probably we must know better the single physiological activities of the present substances before formulating a possible definite conclusion of a general character.

In fact, Hursh, among others, in a study of a large number of wheat plants, observed that a parallelism exists between resistance and acidity as expressed in terms of pH (13). Anthocyanines among pigments are supposed to act as inhibitory substances by virtue of the fact that they are derived from bodies endowed with a high osmotic power.

The alkaloids are inactive, just as are the essential oils, but the latter are quite active *in vitro*. There are, in addition, some investigators who have observed phenomena attributable to true immunological factors. Wagner is one of them. He cites three groups of antibacterial principles (20): (1) The agglutinin that would hinder the movement of cilia. (2) The lysin that would dissolve the membranes of the infecting organism. (3) Other substances, inhibitory to germination of spores.

He also states that the bactericidal factor is strictly connected with the protein fraction of the sap and that it is destroyed by heating 15 min. at 60° C., by oxidases, and peroxidases.

However, let it not be forgotten that constancy and specificity of the reactions are extremely variable. At all events this field seems open to brilliant research work to whoever plans to devote himself with systematic directions. Undoubtedly it is within the microchemical and hystogenic reactions of sap in parasitized plants that we should expect a new light.

So far we have considered only the natural factors of resistance of plants. Let us now turn to the condition of the plant when a host. We have then symbiosis of all possible types, from the most harmless up to the occasionally encountered violent forms where tissues build up physical barriers or where thickly invaded leaves, flowers, or branches are actually shed. Reactions of a physiological nature become evident by the diminishing weight of the dry substance in the diseased leaves through the rise of temperature, as Pavarino says (16), just as in the case of fever in animals. Furthermore, we should recall at this point the theories of Malpighi originally formulated in his classical work, "De Gallis." These theories found favor with a number of Italian and other foreign phytopathologists and were subsequently taken up by Sachs and Beyerinck. To-day there are generally accepted hypotheses of a certain *quid* that stimulates growth as well as cicatrization in plants. Apparently these principles have numerous points of contact with animal hormones or secretions of the endocrine glands. Finally, there has been studied in multiplex forms the phenomenon of phagocytosis of vegetable cells occurring in the roots of many plants, *e.g.*, Orchis. In these cases the germination of seeds is subordinate to the infection brought about by the respective Rhizoctonia. Bernard, using different mycorhizas instead, was able to point out that, while some embryos succumb to the invasion of the mold, others react by means of their cells, limiting the activity of the fungus to a minimum sufficient to guarantee a symbiosis; that is to say, an equilibrium which Bernard calls "*à la frontière de la maladie*" (5).

In addition to these, there is a third case in which the mycelium of the parasite is actually destroyed. The invading hyphae penetrate the embryonic tissues, assume a vegetative character opposed to rapid diffusion,

and proceed to the formation of a clew within the single cells. In the case of the resistant Orchis this clew of mycelium is successively attacked and destroyed. The nucleus of the cells hypertrophies, assuming at times amoeboid aspects; it winds its way through the mycelial mass, resulting in a degeneration of the clew into an amorphous complex; it is sometimes capable of returning very slowly to the primary form. As for the hypothesis of autolysis, one tends to-day to substitute in these cases the interpretation of active cellular factors that operate as directly as the aforementioned nucleus.

The abnormal manner of development of the endophyte within the plant can be related to the phagocytosis, on the one hand, and to the humoral properties of the saps, on the other. This degree of immunity is comparable to that of vaccinated animals.

Indeed, from the tubercles of Orchis, Bernard (6) succeeded in preparing an extract with specific fungicidal and coctible character at 55° C. for 35 min. In concluding what occurs within the Orchis, it is interesting to remember that cellular reactions of a very similar type have been noticed by Demkerly in the cells of a worm in respect to a coccidium. Not only have analogous statements been gathered together recently in the physio-pathological study of several other cases of plant infection in Europe but also a very instructive case has been found on *Arisaema triphyllum* (L.) Schott., a common plant of the woods of New York (11).

To conclude the subject of congenital immunity, let us recall that in cases of wheat plants, subject to *Puccinia graminis tritici* Eriks. & Henn., and of flax, subject to *Fusarium lini* Boll., the presence of immunological factors is made evident under the forms of the so-called hypersensitive reaction characterized by a rapid spreading of the infection, which induces a virulent histological and humoral reactivity that is thoroughly capable of checking the parasite (21).

We now come to the subject of acquired immunity through plant vaccination. Many objections to this have been based on several facts, such as the anatomical and the physiological differences between plants and animals, the usually localized type of vegetable infection, and the supposed lack of a circulation comparable to the circulation of blood in animals, where the immunological phenomena have their principal seat. The circulation of saps is still, for many people, simply a mechanical fact that concerns chiefly the mere lignified vessels without any possible intervention in the delicate processes as associated with immunity. But the most modern views on plant pathology tend to emphasize the meaning of any living cell in the circulation of saps. Concerning the type of infection, let it be sufficient to recall that there are infections in plants due to bacteria that infest the whole organism; also, there are some fungi that spread through cribriform vessels. And do not the most recent researches on animal cells cul-

tured *in vitro* show that they are still capable of producing specific antibodies? There are here, then, no circulatory or nervous correlations; in other words, conditions of life are entirely similar to those of vegetable cells.

Moreover, we can not ignore the importance of the recent studies of Gioelli (12) on the variations of vegetable saps in diseased plants. By means of both chemical and physicochemical analyses and by an extensive application of the refractometric methods, he was able to prove that proteins in diseased vegetables undergo variations analogous to those already observed in diseased animals. Gioelli concluded that "the plant cells react to the infection as the animal cells."

Let us now consider some specific case of vaccination as it has been experimented upon till this very day by several workers. Though these experiments are few and not at all free from criticism, they reveal a definite accomplishment of positive results. Simply on the basis of these first encouraging experiences, we hope for increased interest in this very new and promising field of investigation whose prospects are undoubtedly fruitful.

Carbone (7) lists in a recent paper 18 cases of phanerogamic hosts and cryptogamic parasites whose aspects of immunological phenomena have been checked by various authors. Let us select a few. In 1901 Beauverie (3) observed that when very young Begonia plants are cultivated in the presence of a strain of a weakly-virulent parasite, they resist the attack of *Botrytis cinerea* Pers. Shortly after, Ray (17) stated that when lupines, beans, wheat, and oats are treated previously, either with attenuated cultures of the microorganism or with its extracts, they resist *Bacillus putrefaciens* Ray, which is the common cause of rot, and become slightly diseased for a short time, after which they remain immune from succeeding infections. Zoja (22) obtained similar results by vaccinating wheat against *Helminthosporium sativum* P. K. & B. Acquired immunity was realized by letting the seeds germinate on the aqueous extracts of parasite cultures. After infection, these plants did not show the fungus in any cell. On the other hand, plants germinating in pure water certainly revealed the fungus.

Under different conditions Arnaudi (2) experimented with potatoes by means of a simple technique and achieved similar results. On each side of the potato two cup-like cavities were made, one moistened with the attenuated broth culture of *Bacillus mesentericus* (Flügge) Mig., the other with sterile broth. After 24 hours, at 30° C., Arnaudi scarified both cavities to break the subereous layers and placed in each a loop of the virulent cultures. With variations, dependent upon the degree of the attenuation of the immunizing material, where it has been used, the tissue remained either untouched or only slightly attacked, characterized by a dry circumscribed area not at all comparable to the imposing work of destruction noticeable in the other cavity. In some other work on *Pisum sativum* L., the same author

has shown not only a greater acquired resistance by the prolongation of life of the vaccinated subjects but also the specificity of the immunizing action; in fact, the parallel vaccinations with *Aspergillus oryzae* Ahlb. Cohn and *Blepharospora cambivora* Petri behave quite differently. When the young plants are artificially infected only by the Blepharospora itself they will show a distinct resistance to it, while those vaccinated with the Aspergillus do not reveal any specific resistance to the Blepharospora.

Several other examples can be added to these, such as those recently published by Benigni (4), in Italy, and Nobécourt (15), in France.

The results on the vaccination of plants should be brought together. While the Italian and German workers confirm the presence of a state of high resistance to the infection, the French authors affirm having achieved complete immunity in reinoculation. As far as we know of cases to date, the state of immunity does not seem to last much longer than 1 month. Concerning the nature and the properties of antigens, the first attempts in this field seem to indicate many capital points of contact with the animal antigens, themselves. It is obvious, however, that much, if not the entire field of this specialty, yet remains to be investigated. At all events it seems that we can already detect a decisive action, either of histogenic or of humoral factors.

Finally, there is to be considered the question of superinfection. When a more or less latent infection occurs in an organism, and this is again attacked by the same infectious agent in the same or another part of it, we are referring to superinfection. It may happen that the organism does not react at all to the second infection. This property is distinguished as resistance to the superinfection, and it is essentially one of the types of acquired immunity. Now, the same immunity has been checked, even for plants. Arnaudi, to mention a single name only, working on geranium plants with *Bacillus tumefaciens*, concluded that, out of 13 inoculations into subjects with preexistent tumors, 9 were healed; 4 formed simply tiny tumefactions. The control on 9 inoculations on healthy plants resulted, instead, in 7 cases of tumors and 2 negative cases (2).

There still remain many points to be considered in relation to immunological phenomena in plants. He who desires to increase his knowledge in this field of study, which, furthermore, concerns passive immunity, humoral factors, bacteriophagy, anaphylaxis, and experimental and applied technique, should consult the recent monograph on plant immunity by Carbone and Arnaudi (9). Because of its very thorough discussion on any subject in this field, and of its extensive bibliography, this work is of major importance to that biologist who seeks new perspectives in the study of the pathology of vegetables.

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LITERATURE CITED

1. ALTANA, G. La infezione vaccino-vaiolosa e la sua eziologia. Rivista d' Igi. e Sanità pubblica **23**: 436–450. 1912.
2. ARNAUDI, C. Sull' immunità acquisita nei vegetali. Atti Soc. Ital. Sci. Nat. & Mus. Civico Stor. Nat. Milano **64**: 230–238. 1926.
3. BEAUVERIE, F. Essais d'immunisation des végétaux contre les maladies cryptogamiques. Compt. Rend. Acad. Sci. (Paris) **133**: 107–110. 1901.
4. BENIGNI, E. Note sull' *Ustilago maydis* (D.C.) Corda nella Valle Padana. Riv. Patol. Veg. **17**: 57–72. 1927.
5. BERNARD, N. Remarques sur l' immunité chez les plantes. Bull. Inst. Pasteur **7**: 369–386. 1909.
6. ————. La culture des orchidées dans ses rapports avec la symbiose. Jour. Soc. Nat. Hort. France, Ser. 4, **24**: 1923.
7. CANTACUZÈNE, J. Le problèm de l'immunité chez les invertébrés. Soc. de biologie, Célébration du 75me Anniversaire de la fondation de la Société. Pp. 48–119. 1923.
8. CARBONE, D. Ueber die aktive Immunisierung der Pflanzen. Centralbl. Bakt. Abt. II, **76**: 428–437. 1929.
9. ————, and C. ARNAUDI. L' immunità nelle piante. 274 pp., Stucchi Ceretti. Milano, 1930.
10. DE KOCK, G. v. D. W. A contribution to the study of the virus, haematology and pathology of infectious anaemia of equines under South African conditions. Union So. Africa Dept. Agr. 9th–10th Rpt. of Dir. Vet. Ed. and Res. **1923**: 253–313. 1924.
11. DUFRENOY, J. Étude cytologique des rapports entre parasite et cellule végétale. Ann. Inst. Pasteur **43**: 218–222. 1929.
12. GIOELLI, F. Alcune determinazioni rafrattometriche in succhi di piante ammalate. Atti Ist. Bot. R. Univ. Pavia, III **3**: 49–58 (1926). 1927.
13. HURSH, C. R. Morphological and physiological studies on the resistance of wheat to *Puccinia graminis tritici* Erickss. & Henn. Jour. Agr. Res. **27**: 381–412. 1924.
14. METALNIKOFF, S. I. L' infection microbiènne et l' immunité chez la mite des abeilles (*Galleria mellonella*). Paris, Masson et Cie. 1927.
15. NOBÉCOURT, P. Contribution à l' étude de l' immunité chez les végétaux. 176 pp. J. Barlier & Cie, Tunis. 1928.
16. PAVARINO, G. L. Intorno alla produzione del calore nelle piante ammalate. Atti Ist. Bot. R. Univ. Pavia, II **13**: 355–384 (1910). 1914.
17. RAY, J. Cultures et formes atténuées des maladies cryptogamiques des végétaux. Compt. Rend. Acad. Sci. (Paris) **133**: 307–309. 1901.
18. STAKMAN, E. C. A study in cereal rusts; physiological races. Minn. Agr. Exp. Sta. Bul. 138. 1914.
19. VAVILOV, N. Immunity of plants to infectious diseases. Ann. Acad. Agron. Perov. 1918. Moscow, 1919.
20. WAGNER, R. J. Ueber bakterizide Stoffe in gesunden und kranken Pflanzen. Centralbl. Bakt., Abt. II **42**: 613–624. 1914.
21. ZIMMERMANN, A. Sammelreferate über die Beziehungen Zwischen Parasit und Wirtspflanze. 2. Die Uredineen. Centralbl. Bakt., Abt. II **65**: 311–418. 1925.
22. ZOJA, A. L' immunità nelle piante. Atti Ist. Bot. R. Univ. Pavia, III **2**: 15–47. 1925.

PHYTOPATHOLOGICAL NOTE

A correction.—In our paper entitled "Stripe rust, *Puccinia glumarum*, on wheat in Argentina" (Phytopathology **20**: 981–986, 1930) there appears on page 981 the following statement: "Elsewhere, in South America, it [*P. glumarum*] apparently had never been collected until October, 1929, when the junior writer [Richard O. Cromwell] observed it in . . . Argentina." Our attention has since been called to a published account[1] of an earlier observation and identification of this rust in Argentina by W. Rudorf, who collected it also in October, 1929, evidently only a few days before it was observed by the junior writer.—Harry B. Humphrey and Richard O. Cromwell.

[1] Recientes análisis de trigos hacen prever una infección de "Puccinia glumarum tritici." La Razon, Agosto 22, 1930. Buenos Aires.